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HAJIME MASAMUNE
Professor Emeritus of the Tohoku University

Professor and Director of the Cancer Research Institute of the Tohoku Pharmaceutical College

Professor Hajime Masamune suddenly passed away from apoplectic stroke at his home in Sendai on October 19, 1959, at 63 years of age. His unexpected death shocked and grieved all who knew him.

Prof. Masamune was born at Bizen-machi (formerly called as Iri-mura), Okayama prefecture, Japan, on January 6, 1896, as the first son of Dr. Hosaburo Masamune, a physician, and of his wife Shizue. After receiving his early education at his home place, he entered the Kyushu Imperial University

(now the Kyushu University) School of Medicine in Fukuoka and graduated from the school in March, 1921. Next month of his graduation, he was admitted to the department of medical chemistry of the same school as an assistant in biochemistry to Prof. M. Goto. In 1927, he obtained the degree of Doctor of Medicine through his work on the mechanism of fluctuation of blood sugar level after administration of pilocarpine and other related compounds. In December, 1928, he was raised to an assistant professor of the same medical school and soon after that, he went abroad to make advanced studies in biochemistry. At first, he joined Prof. J.B. Conant's group in the organic chemistry laboratory of Harvard University and stayed there for about a year. In 1930, he moved to Germany and engaged in research on carbohydrate chemistry under Prof. B. Helferich in the organic chemistry laboratory of Leipzig University. He returned to the Kyushu University in 1931. In 1934, he was appointed to the Professor of biochemistry of the Hokkaido Imperial University (now the Hokkaido University) and held the post until 1942, when he moved to the Tohoku Imperial University (now the Tohoku University) as the professor of biochemistry of the medical school. He retired from the Tohoku University in March, 1959, and next month he was conferred with the title of Professor Emeritus of the Tohoku University. Same month, he was appointed to a professor and the director of the Cancer Research Institute of the Tohoku Pharmaceutical College in Sendai. He kept the position through the day of his death.

Prof. Masamune's work on aminosugars and mucopolysaccharides was originated early in his days in Germany when practically nothing had been known about the chemistry of these compounds. After he returned to his home country, he focused his whole efforts on the study in this field. Studies on glucuronidase was his first work in the field, then he turned to the structure of chondroitin sulfate and succeeded in determining its real molecular structure. Works done thereafter by Prof. Masamune and his group could be classified into the following three main groups. (1) Isolation, purification, and components analysis of various mucosubstances (mucopolysaccharides, glucidamins, glycoproteins, mucoproteins, mucolipids, etc.) from human and animal sources. Physicochemical and immunochemical studies were also made on properties of these substances. On the numerous results from their broad investigations of these mucosubstances, Prof. Masamune came to establish an unique system of classification of the mucosubstances. (2) Investigations of the chemical structure of blood group substances. Particular attention was paid to clarify the chemical nature of determinant groups of blood group activity on the consideration of chemical components and structure of blood group substances. On this line, chemical synthesis of blood group active mucolipids was also attempted with appreciable results. (3) Comparison of mucosubstances from human cancerous tissues with those from corresponding normal tissues. Significant differences

in chemical and physicochemical properties of mucosubstances were found between cancerous tissues and normal tissues. Through these studies, it was evidenced that cancer tissues are biologically immature by nature. Also his studies on K.I.K. factor and toxohormone added valuable findings to the knowledge of cancer problems.

Besides those mentioned above, there have been many significant accomplishments done in Prof. Masamune's laboratory. There were studies, for instance, on pathogeny of toxemia of pregnancy. The number of original reports published with his name and/or his pupils' names exceeded three hundreds. His earlier reports appeared mostly in J. Biochemistry (Japan). The papers prepared at the Tohoku University were published in the Tohoku J. Exp. Med., to which he had served as one of the editors since 1950. His sincere efforts devoted to the Journal have been highly appreciated by all who are concerned with the Journal.

In 1955, Prof. Masamune attended the Third International Congress of Biochemistry held at Brussels and read before the Meeting a paper in which essentials of his recent studies were condensed. After the Meeting, he traveled around Europe and the United States, visited laboratories and had discussions with many scientists of similar research fields. He appeared very content with the travel. Particularly he was pleased to have known through the travel that his basic chemical investigations on mucosubstances had been estimated properly. He was convinced that his work had provided effective clues for the understanding of biologically active mucosubstances.

Prof. Masamune was one of the most active and responsible leaders of the Japanese Biochemical Society. During the year of 1954, he was the president of the Society.

He published, with Prof. K. Kodama, now the President of the Tokushima University, two text books of biochemistry. One of which, *Medical Chemistry*, was first published in 1939 and thereafter ten editions of it appeared by now.

Prof. Masamune was a man of hard work. He loved to work and enjoyed laboratory life. He had always many ideas in his mind and was brave to construct speculations on the experimental results so far obtained at any step of the work. He liked to discuss his ideas and speculations with his laboratory members and by this way he stimulated creative spirit of his pupils. He was strict as far as it concerned with science, but out of science, he was quiet, gentle and warm hearted to any person. His nice personality influenced on many who were acquainted with him.

Prof. Masamune got married with Miss Chikako Kondo in 1919. Miss Kondo, now Mrs. Masamune, was a classmate of Prof. Masamune at the primary school. He and she were two outstanding pupils in the class and love had grown naturally among them which later fruited in their marriage. The

Masamune's home life had been filled with light. They got five sons and a daughter and now two of the sons live with Mrs. Masamune in Sendai.

Now we have lost Prof. Masamune, a prominent biochemist and a respectable personality. We recall his life devoted to science and humanity and we mourn him deeply.

GORO KIKUCHI
ZENSAKU YOSIZAWA

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Interaction between Succinylcholine and Imbretil

By

Kenichi Iwatsuki and **Mamoru Murakami**

Department of Anesthesiology, Tohoku University School of Medicine, Sendai, Japan

(Received for publication, November 25, 1959)

Imbretil (hexamethylene-bis-carbaminoylcholine bromide) was first introduced in clinical practice as a valuable, long acting muscle relaxant by Brücke and others[1)2)3)] after a wide investigation on the pharmacological actions of certain polymethylene-bis-carbaminoylcholines by Klupp, Kraupp, Stormann and Stumpf.[4)] It has been reported that this agent is biphasic in its mode of action, namely it produces at first a depolarization block, which changes rather rapidly into a curare-like, non-depolarization block. The latter is antagonized by neostigmine.[5)6)7)] The authors have used this relaxant in more than 150 cases with satisfactory results. The action of Imbretil being cumulative following its repeated administration, it is advised to give it in one portion and if necessary, to supplement muscular relaxation by short acting relaxants such as succinylcholine. But some authors[1)2)11)] drew attention to the fact that succinylcholine was potentiated when it was administered after Imbretil. This was also confirmed by the authors in dogs as well as in clinical cases. From the generally accepted reciprocal interaction between a depolarizer and a non-depolarizer it is rather expected that succinylcholine is antagonized after Imbretil, as shown after d-tubocurarine chloride. Therefore it seems difficult to explain this phenomenon from this mechanism. Some experimental results are presented in the present paper to offer an explanation to this phenomenon.

METHODS

Mongrel dogs were used in the experiments. After intramuscular anesthesia with nembutal (25–30 mg./kg.) tibial muscle contraction elicited by the supramaximal electric stimulus applied to the innervating nerve was recorded on a smoked paper drum. The electric stimulus was applied every 10 seconds. The action of succinylcholine chloride was compared before and after the administration of Imbretil. The second dose of succinylcholine was given after the action of Imbretil was almost disappeared. The effect of vagostigmine (neostigmine) or d-tubocurarine chloride was observed when it was given during

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the action of succinylcholine after Imbretil.

RESULTS

Fig. 1 shows the action of succinylcholine chloride before and after the administration of Imbretil (0.015 mg. /kg.). The action of succinylcholine was

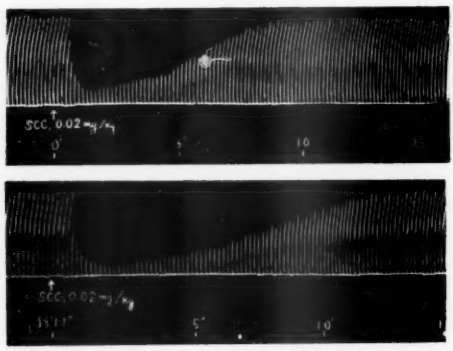

Fig. 1. The action of S.C.C. after Imbretil (Dog 13 kg.)
A: S.C.C. — Control
B: S.C.C. after Imbretil (0.015 mg./kg.)
Note that the action of S.C.C. is potentiated after Imbretil.

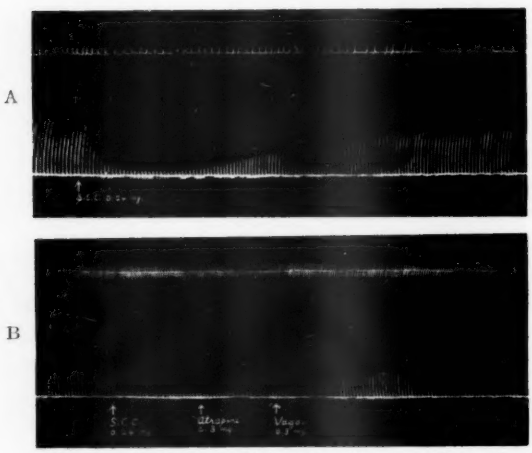

Fig. 2. The effect of Vagostimgine upon the action of S.C.C. after Imbretil (Dog 12 kg.)
A: S.C.C. — Control
B: An antagonizing effect of Vagostigmine upon the action of S.C.C. after Imbertil (0.015 mg/kg.)

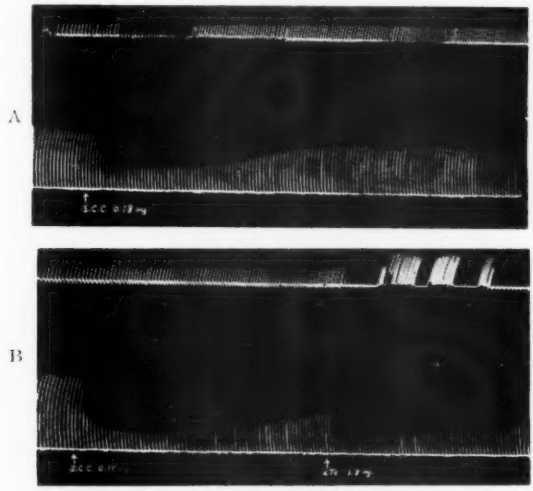

Fig. 3. The effect of dTc upon the action of S.C.C. after Imbertil (Dog 12 kg.)
A : S.C.C. — Control
B : A synergistic effect of dTc upon the action of S.C.C. after Imbertil (0.015 mg./kg.)

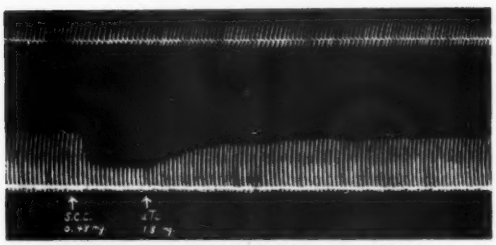

Fig. 4. The effect of dTc upon the action of S.C.C. (Dog 16kg.)
Note that the action of S.C.C. is antagonized by dTc.

definitely potentiated after Imbretil. Fig. 2 shows the effect of vagostigmine (0.025 mg./kg.) and Fig. 3 shows the effect of d-tubocurarine (0.15 mg./kg.) upon the action of succinylcholine after Imbretil. Vagostigmine antagonized, while d-tubocurarine potentiated the action of succinylcholine. Succinylcholine chloride is primarily antagonized by d-tubocurarine chloride as shown in Fig. 4. These results suggest that the mode of action of succinylcholine may be changed from its original depolarization block to curare-like, non-depolarization block due to the previous administration of Imbretil.

DISCUSSION

Potentiation of the action of succinylcholine after the administration of Imbretil has already been observed by Brücke, Reis[1)3)] and Holzer[2)], who, however, did not refer to its mechanism. It was reported by Klupp and others that Imbretil depressed the activity of plasma cholinesterase, therefore there may be a possibility that it potentiates the action of succinylcholine as do the other cholinesterase inhibitors. But from this point of view it is difficult to explain the antagonizing effect of vagostigmine and the synergistic effect of d-tubocurarine upon the action of succinylcholine after Imbretil. If it is allowed to assume a hypothesis that previously administered Imbretil may affect the sensitivity of the end-plate and predispose the response to succinylcholine to a non-depolarization block, these phenomena can be reasonably explained. Recently we have experienced cases, in which prolonged respiratory depression that happened to occur following the administration of ordinary doses of succinylcholine after Imbretil was strikingly improved by Tensilon. This fact shows that an altered response to succinylcholine can occur after Imbretil not only in dogs, but also in anesthetized men. It has been recognized that depolarizing agents such as decamethonium and succinylcholine are in itself biphasisc in their mode of action,[8)] which is likely to be affected by various factors[9)] such as the prolonged administration of the agents or certain pathological states of the patients. Hodges and his collaborators[10)] reported that succinylcholine produced an atypical, cuare-like block when it was used after the intravenous infusion of oxytocin – a posterior pituitary extract and they suggested that such an atypical response may be in part due to some imbalance of potassium ions following oxytocin infusion as in case of the prolonged administration of depolarizing agents. Imbretil is believed to be biphasic in its mode of action, producing a depolarization block at the initial stage, and potassium release is also observed following its adminstration.[11)] Therefore it seems not incredible that the previous administration of Imbretil may affect the sensitivity of the end-plate to depolarizing agents in certain extent, but its mechanism in detail is further to be studied.

SUMMARY

Experimental studies were carried out in dogs to investigate the interaction between succinylcholine chloride and Imbretil. The results revealed that the previous administration of Imbretil potentiated the action of succinylcholine, which was, however, antagonzied by vagostimgnine and was synergistic with d-tubocurarine. This fact suggests a possibility that Imbretil may enhance the transition of the mode of action of succinylcholine from depolarization block to non-depolarization block.

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Experimental Studies on Homologous Heart Transplantation

First Report: Part I. Techniques of the Transplantation Part II. The Behavior of the Transplanted Heart

By

Fumio Jin

From the Surgical Clinic of Prof. S-T. Katsura, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, November 25, 1959)

The experimental investigation of the homologous heart transplantation would seem to be a most valuable object for study not only of transplantation of organs with highly specialized tissue but also of the physiologic and pharmacologic properties of the heart. Mann, Priestly, Markowitz, and Yater published a pioneering report in 1933, in which they worked with the method of so-called cervical heart transplantation; vascular anastomoses were created between the recipient's neck vessels and the puppy heart vessels so as to perfuse the coronary circuit of the puppy heart in vivo. Since then, there are a few reports in the literature on this problem, but they treated for the most part the fate of the transplanted heart without establishing a good method of vascular anastomosis as a fundamental problem of the transplantation. This investigation has been carried out in order to find the most reliable method of the cervical heart transplantation by experimental evaluation of the early transplantation failures and also to investigate the general behavior of the transplanted heart from the electrocardiographic and pathological point of view.

I. TECHNIQUES OF THE TRANSPLANTATION

Experimental Methods

The following principles of procedure were found to be useful for success.

1. To prevent the possibility of intra-cardiac clotting the transplanted heart should be completely heparinized while it is being removed from the donor's body.

2. Each pulmonary vessel must be individually ligated so as to prevent large bleeding due to insufficiency of mass ligation.

3. Precaution against dilatation of the right heart must be taken by keeping

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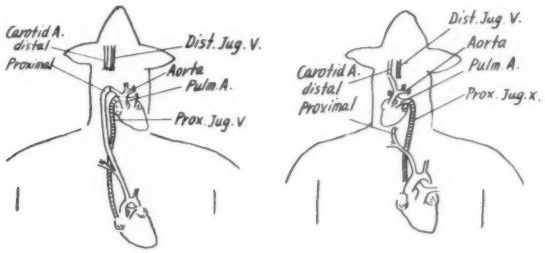
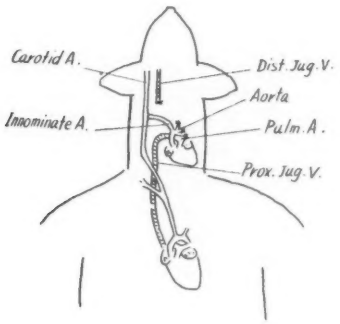
good coronary venous outflow via the pulmonary artery under careful positional consideration of the transplanted heart.

Adult mongrel dogs weighing 8 to 19 kg. were used as the recipients. Healthy mongrel puppies weighing 1 to 3 kg. served as heart donors. The essential procedure was to establish coronary circulation of the transplanted heart by anastomosing its aorta to a suitable vessel of the recipient. From the results of preliminary experiments, three technical methods were developed: In first



Group I Group II

Fig. 1. On the left, the schema shows transplantation in Group I, and on the right, in Group II,



Group III

Fig. 2. The schema shows the transplantation of Group III.

group, the coronary circulation was established by ansatomosis between the proximal end of the carotid artery and the aorta, in the second group anastomosis of the distal end of the carotic artery with the aorta was effected, and

finally in the third group the end of the aorta and the side of the carotid artery were anastomosed.

All operative procedures were carried out under endotracheal ether anesthesia with aseptic technic. The preparation of the recipient neck was as follows: A large skin incision was made along the sternocleidomastoid muscle on a side of the recipient neck, then the carotid artery and the jugular vein were cut free. The adventitia was carefully strippped from the vessels for anastomosis, then these vessels were cut between clamps at about 4 cm above the suprasternal notch except for the third group in which the carotid artery was preserved without cutting. In the first group, the distal end of the common carotid artery was ligated while the proximal end was prepared for anastomosis. In the second group, the opposite was done: The proximal end of the dissected common carotid artery was ligated and the distal end was reserved for anastomosis. The jugular vein was always ligated on the distal dissected end and the proximal end was prepared for anastomosis in each group. Meanwhile a puppy was anesthetized by means of open drop ether, and the chest was opened by mid-sternal splitting incision. And then the brachiocephalic artery, the left brachial artery, the azygos vein, the superior vena cava, the inferior vena cava and the descending aorta were doubly ligated and cut between the ligatures. When the last two vessels were tied, 5 mg. of heparin was injected into the right atrium in order to prevent intra-cardial clotting. The heart with lungs was now removed after dissection of the bronchus, then the pulmonary arteries and veins were carefully cut between the ligatures. The heart was then ready to be anastomosed to the previously prepared vessels of the recipient dog in the described manner; for instance, the aorta or the brachiocephalic artery was anastomosed to the proximal end of the carotid artery, and the pulmonary artery or its branch was anastomosed to the proximal end of the jugular vein in the first group. The anastomosis of the vessels may be accomplished utilizing the femoral artery and vein, the common carotid artery and the external or internal jugular vein, or the common iliac artery and vein. At the anastomosis, two per cent solution of sodium citrate was applied to prevent blood clotting. Following anastomosis, the clamps were released first on the venous side and then on the arterial side. Care should be taken not to kink the venous side anastomosis, and if neccessary, the translpanted heart was anchored by a suture to the surrounding tissues. The wound was closed after establishment of regular cardiac rhythm of the transplanted heart. Electrocardiographic records were taken on the recipient neck just above the heart in all experiments and blood pressure was recorded with a by polyvisocardiogram "Sanborn" in some occasions.

Results — Table 1 and 2 show the results. The heart begun to start in all instances with normal sinus rhythm following temporarily auricular or ventricular fibrillation for the period of 2 to 3 minutes immediately after anastomosis was

TABLE I. Results of Homologous Canine Heart Transplantation

No.	Total circulatory cessation of the heart	Heart rate immediate. after graft	Survival of the heart	Cause of death and notes
	min.	min.		
1	89	64	2 hrs	Intra-cardiac blood clots were found. No heparin was used
2	90	110	2 hrs	Rupture of the left atrium
3	—	—	1 hr	Rupture of the left atrium Artifiicial atrial septal defect was attempted
4	65	80	13 hrs	Recipient was expired by unknown cause
5	55	160	5 min.	Ruputre of the left auricle
7	—	—	30 min.	Thrombosis at anastomosed area
		Group II		
6	60	—	12 hrs	Recipient died of traumatic bleeding. Before death recipient sacrificed
8	—	—	30 min.	Thromobsis Femoral artery was used
9	65	80	130 hrs	Recipient died of bleeding from traumatic transplanted heart
12	—	—	0	Coronary air-embolism. Ventr-fibrill.
15	55	—	15 min.	Coronary air-embolism
16	—	90	3 hrs	Recipient died of unknown cause
17	—	54	81 hrs	Kenacort (a Corticoid) was used
18	—	—	30 min.	Venous side thrombosis
19	53	—	12 hrs	Autopsy was made
20	65	—	80 hrs	Recipient injured the transplant.
24	50	—	25 hrs	Autopsy was made
25	—	86	30 hrs	Autopsy was made
26	60	80	15 hrs	Recipient died at 15 hrs suddenly
		Group III		
10	75	—	120 hrs	Autopsy showed wide myocairdal necrosis
11	—	—	2 hrs	Coronary air-embolism
13	50	92	80 hrs	Recipient died of traumatic bleeding from transplanted heart
21	60	60	100 hrs	Autopsy was made
22	45	—	2 hrs	Recipient died of pulmonary embolism
23	50	78	85 hrs	Trauma
27	—	80	—	Sacrificed after blood pressure measuring
28	50	80	—	and x-phot.
29	50	100	70 hrs	Autopsy was made
Average	min. 59	min. 83	hrs. min. 23.52	

TABLE II. Causes of Eary Transplantation Failure
(Survival of transplanted heart 0 to 10 hours)

Cause of failure	I Group	II Gr.	III Gr.
Intra-cardiac cloting	1	0	0
Thrombosis	1	2	0
Left atrial rupture	3	0	0
Coronary thrombosis	0	2	1
Unknown	0	1	1
Nos. of failure/nos. of xperiments	5/6	5/14	2/9

accomplished (Fig. 6), and, the time required for the anastomosis to consummate, or in other words, the total time of circulatory cessation of the grafted heart ranged from 45 to 90 minutes. The heart rate was not identical in all instances and showed a wide variation of 54 to 160 per minute.

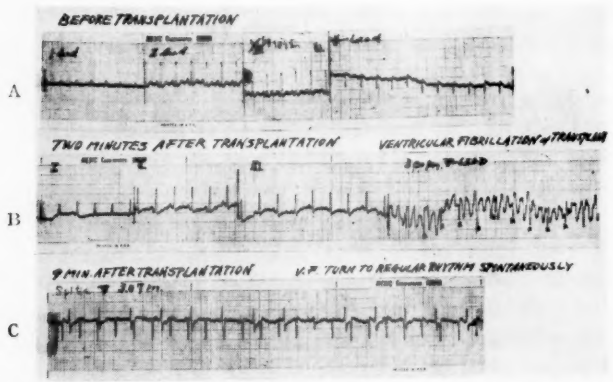


Fig. 6. Electrocardiographic tracing of a common course of the transplanted heart. A. : Preoperative control tracing, showing lead I, II, III and V from the site to be grafted. B : 2 min. after transplantation, on the V-lead ventricular fibrillation of the transplant is shown. C : 7 min. after the ventricular fibrillation; the upward deflections of the electrocardiographic complex show that the transplant's ventricular fibrillation has been turned spontaneously to a regular rhythm.

Twelve cases of all 29 experiments failed the cardiac function of the transplant within 10 hours of the transplantation, these cases were called as early transplantation failure and these causes were listed as follow; thrombosis at the anastomosis, coronary air-embolism and rupture of the left auricle or atrium in 3 cases repspectively, and two of unknown cause, and one of intra-cardiac clotting. The cause of thorombosis and coronary air-embolism were shown in each groups, but the rupture of the left atrium indicated only in the first group. It was thought that the rupture may result from high blood pressure of the carotid artery, which was anastomosed. The pressure were recorded from the anastomosed area and the left auricle as Fig. 3 : In the first group, the anastomosed area showed 100 to 120 mm Hg and the auricular pressure was increasing immediately after release of the clamp at the carotid artery to 120 mm Hg and more, and finally the rupture was induced. In the second group, the pressure of anastomosed area showed 70 to 75 mm Hg and intra-auricular pressure remained at about 5 to 15 mm Hg. throughout. In the third group, the pressure of the anastomosed area remained constant at 90 to 100 mm Hg and the intra-

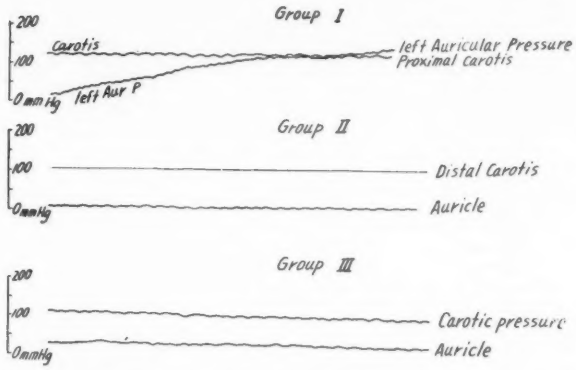


Fig. 3. Pressure tracings presented schematically in each group. Group I: Pressure at anastomosed carotid artery shows 100 to 120 mmHg. constantly, while that of the left auricle increases more than this. Groups II & III show stable pressures.

atrial pressure indicated 5 to 20 mm Hg (Fig. 3).

Roentgenographies revealed clearly that the blood flowed back to the left ventricle or the atrium from the carotid side of the anastomosis in the first group, whereas in second or the third group the coronary circulation was clearly found to show no stagnation or regression of blood in the left side ventricle or the atrium. (Figs. 4 & 5)

Fig. 4. X-ray picture of transplant in Group I. Injection of Urocoline is showing stagnation and back-flow to the left atrium.

Fig. 5. X-ray picture of transplant in group III. Showing clear coronary flow and no stagnation of blood.

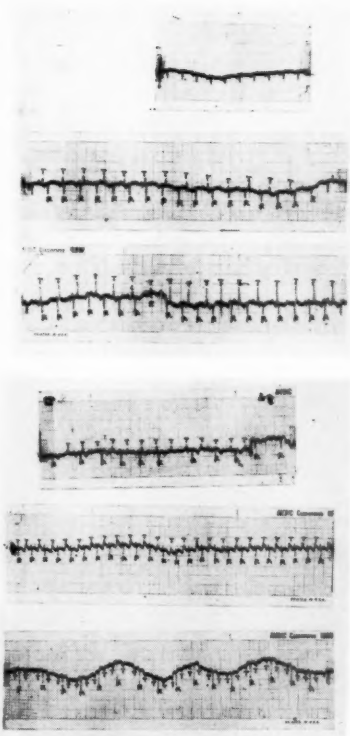
Only too few reports on the techniques of transplantation have been published and only shortening of the time required for anastomosis and preventative measure of blood clots have been emphasized in these reports.[1)2)3)] Because of the intra-cardial blood clotting, all preliminary experments failed and complete heparinization was necessary in the heart transplantation. 5 mg. of heparin was injected into the heart at time of removing the puppy's heart and the results obtained were quite satisfactory in these experiments. Shortening of the time required for the anastomosis was another problem and stressed for a common reason, that is; long circulatory cessation might induce irreversible myocardial damage. Though this problem will be discussed by the author's experimental work later, the time was surprisingly long beyond presumable limitation and as a matter of fact the transplanted hearts recovered well in all experiments even though the time required was about 59 minutes for the anastomosis. Only one case out of 29 experiments did not revive on occount of coronary air-embolism. These results were seemingly supported by report of Webb et al[1)] that no myocardial damage was induced within 90 minutes of circulatory cessation under normothermic condition. The method adopted in my first and second groups for additional artificial atrial septal defect and other methods were reported and their results were not uniform. Early transplantation failure in this study were investigated, and it was found that in the first group rupture of the left atrium was caused by excessive blood pressure of the anastomosed vessel and actually left atrial rupture developed in three cases. Preventional procedure of stagnation of blood in the atrium was imposed by artificial atrial septal defect but the result was not satisfactory. In the second group the result was good but a case failed by too low blood pressure presumably. In the third group no untoward event occurred and the method employed here may be recommended as a simple and controllable transplantation method, *i.e.* the blood pressure may be controlled by narrowing the carotid artery in some degree if neccessary. After all, the last method is recommended as a safe technique.

II. BEHAVIOR OF THE TRANSPLANTED HEART

Method — The experimental material was the same as described in the previous section. ECG of V-lead were taken as a control in the recipient's neck prior to the grafting and after the transplantation. Following the transplantation the recipient was sacrificed in 12 hours, 3 days, 4 days, 5 and 6 days respectively and the transplanted heart, the recipient's heart, liver, kidney, spleen, and adrenal gland were immersed in 10% formalin solution and microscopic studies were performed following hematoxylin or Van Gieson's staining.

Results — ECG of V-lead on the recipient's neck recorded two independent cardiac cycles, and the two different types of QRS complex showed regular rate and appeared in mutually refractory periods.

ECG V-lead over neck of recipient



Time	Heart rate	
	Transplant	Recipient
Before transplantation	—	160
1 hour after transplant	135	170
20 hours after transpiant	150	164
49 hours after transplant	189	156
100 hours after transplant	214	190
120 hours after transplant	300	130

T : Transplant's
R : Recipient's

Figs. 7. & 8. Electrocardiographs of a 130 hours surviver. The major deflections of the electrocariographic complexes are showing the recipient ventricle complex, while the minor complexes originating from the transplanted heart.

Generally speaking, QRS complex of the transplanted heart showed almost the same pattern in the course of post-transplantation, so a case of 130 hours after transplantation is shown in Figs. 7 & 8: The recipient's heart rates remained at around 160 per min. before and after transplantation while the transplanted heart beat 135 one hour and 189 at 49 hours after the transplantation and the rate of the transplanted heart become faster to reach 300 at 120 hours after the transplantation. Besides a shortening of the R-R interval of the transplant, the P wave and the QRS complex gradually indicated low voltage and finally the former disappeared 49 hours later. The QRS intervals were lengthened 100 hours after the transplantation but still the regular rhythm

of the transplant was remained.

Gross pathology: One to two days after the transplantation, the pericardium was covered by rigid fibrostic granulomata and the myocardium was apparently normal but sometimes tiny hemorrhages were noticed in the endocardium and thrombi were proved in the right atrium on some occasions. Three days or more after the transplantation, suppurative exudation came out from the wound, the granulomata became necrotic and deposition of the exudation was demonstrated in the surrounding of the pericardium. In the myocardium small necrosis or hemorrhages were noticed here and there. On the fourth and the fifth days the transplanted heart were showed the above findings much more extensively. On the sixth day the transplant, esp. the myocardium, became yellowish gray-white and the pericardium was swollen and mottled.

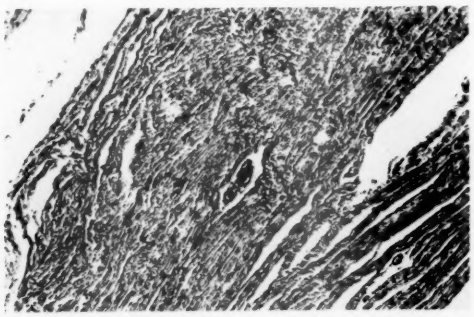


Fig. 9. Photomicrograph of myocarcardium 24 hours after transplantation. Small thrombus is shown in the coronary vessel. Though there are slight edema and necrobiosis, cell invasions are very slightly present. H.-E. stain.

Microscopic findings: One to two days after the transplantation the pericardium was invaded by chiefly polynuclear leucocytes and partly by plasma cells. In the myocardium, perivascular edema and histiocyte infiltration were found but it appeared almost normal except that a few muscle fibers were necrobiotic. Relatively fresh necrosis was noticed at the top of the papillary muscle (Fig. 9). On the third day some suppurative findings were in the pericardium and plasma cell invaded along the coronary vessels, in the myocardium edema and necrosis were found, resembling Ashoff's Knoten, that is; Localized basophile cell infiltration by giant cells was noticed. (Figs. 10 & 11). On the fourth to the fifth day, hemorrhagic exudation became more extensive and the entire myocardium was edematous with basophile cell and fibroblastic cell infiltration and perivascular change were distinct. The endocardium revealed suppurative granuloma with perivascular change and partially developed Ashoff's

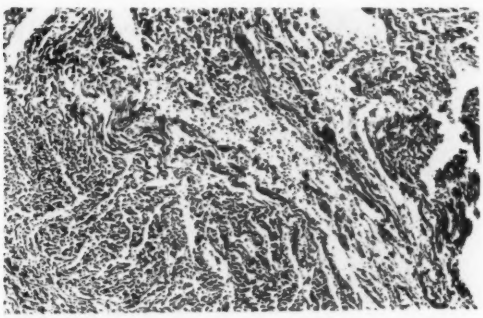


Fig. 10. Photomicrograph of outside of myocardium at 3 days after transplantation. A small nodule consisted of basophile large cells and fibroblasts is shown in the left upper corner. H.-E. stain.

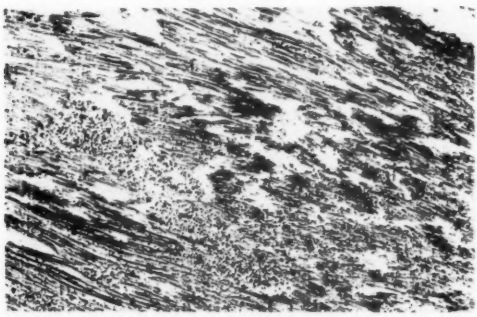


Fig. 11. Photomicrograph of mid-zone of myocardium at 3 days after transplantation. Entensive necrosis and cell inveasions are shown. H.-E. stain.

Knoten (Figs. 12 & 13).

No gross pathologic findings were found in the recipient's organs, but some microscopic findings were as follows: Heart: On the fifth day, very slight swelling in the endcardium.

Liver: On the fourth and the fifth days, plasma cell or leucocyte infiltration in the mesenchyma and in a part of the parenchyma was present.

Spleen: On the third, fourth and fifth days, edema and hyperaemia, and slight perifollicular plasma cell, reticulocyte and eosinophile cell infiltration.

Kidney: On the fifth day, edema and slight tubular dilatation, and thickening of the epithelium were present.

Adrenal grand: On the third day fat diffused in the fascicular zone and thicken-

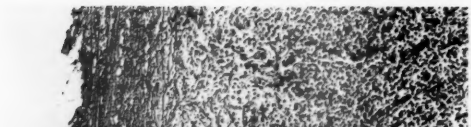


Fig. 12. Photomicrograph of surrounding epicardium at 5 days after transplantation. Very strong suppurative granuloma with cell invasions is shown. H.-E. stain.

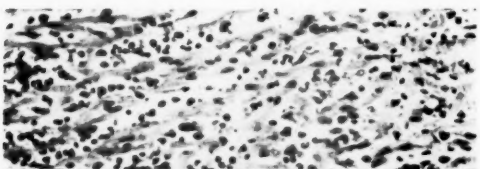


Fig. 13. Photomicrograph of magnifying above granuloma is showing mainly histiocytes and polynuclear leucocytes.

ing of the cortex and swelling of the glomerular zone were present on the fifth day.

Among 29 experiments, 3 cases of survival for 130 to 100 hours, 5 cases of 100 to 50 hours and 7 cases of 50 to 10 hours were obtained in this study. ECG of the transplant was almost the same as those obtained by Makus,[3] Wesolowski,[5] Matejicek,[6] et al. The ECG of V-lead of the neck above the transplanted heart revealed two types of QRS complex, namely, one was of the recipient's heart and the other was of the transplant. With lapse of time after the transplantation, shortening of the R-R interval and low voltage were observed reflecting some pathological changes of the transplant. In the recipient heart no pathological findings was found and those of the transplant might have been caused due to pathological changes such as extensive suppurative exudation surrounding the pericardium or myocardial damages, while ECG of the

recipient's heart was normal without any myocardial damages. In the pathological study, the transplant revealed chiefly suppurative change of the pericardium and very slight change of the myocardium, esp. perivascular edema or plasma cell infiltration in one or two days. In three days, the transplant showed clearly some necrosis in the myocardium and formations like Ashoff's Knoten, and also the perivascular change became more extensive. After the fourth day, transplant showed aggravation of the above mentioned findings. On the sixth day, the transplant revealed large wide spread myocardial necrosis and the function of the heart was finally destroyed. Microscopic study of the recipient's organs showed some pathologic findings of suppuration and definite specific findings of the transplantation.

COMMENT

Reports on homologous heart transplantation have been not many in the literature,[1)2)3)5)8)9)] and these communications discussed chiefly the behavior of the transplanted heart, few of them touching on the techniques of the transplantation. Three methods of the transplantation were adopted in this series of studies. Twenty nine experiments were performed after several preliminary studies and all experiments but one showed that the transplanted heart recovered normal sinus rhythm followed by temporary atrial or ventricular fibrillation. On these occasions, a problem came to us, namely, early transplantation failure by desuetude of the heart within 10 hours transplantation and the failures were listed in each groups (Table 1); 5 of the 6 cases of the first group showed early failure and 3 cases were listed as rupture of the left atrium, 5 of the 14 cases of the second group, 2 of the 9 cases of the third group were in the early failure cases respectively. Measurement of blood pressures in each group clearly showed that blood regression from the anastomosed vessel caused the atrial rupture in the first group. A report[3)] described another procedure of artificial atrial septal defect and the author tested this procedure in two cases but the result was not satisfactory. The second group showed good results but one case failed presumably on account of low blood pressure. The third group showed best results among all the groups, and the method is recommended as a safe transplantation method because of its simplicity and controlability; if high blood pressure was obseaved, narrowing the carotid artery can easily control regression of the blood. Markowitz,[1)] Luisada,[2)] et al. emphasized the importance of shortening the time of total circulatory cessation and viperfusion technique was proposed, but the results were not so good as Weslowski's, Fennesey's[5)] or the author's. Though Crowell[7)] stated that total circuit cessation induced multiple micro-thorombus in vessels, this study with heparin used showed no disturbances in cessation for 59 minutes. Concerning the behavior of the transplanted heart, previous papers[1,2,3,5] were reviewed and the transplant has been said to fail in

about one week. In this study, 3 cases of survival of 130 to 100 hours, 5 cases of 100 to 50 hours and 7 cases of 50 to 10 hours were obtained. ECG findings of the transplant showed that two independent P wave QRS complexes were in regular rhythm and as time elapsed, low voltage or R-R shortening was noticed. On the pathological findings of the transplantation, since the report by Mann, Priestley[1] as pioneers, Luisada,[3] Wesolowski,[5] and others have reported and the author's experiment showed in 6 days the Initial Take byMe dawar.[10,11] As a cause of the heart-transplantation failure, anoxia during the total circulatory cessation was discussed already but is not enough in itself to constitute the mean cause of the failure. Next, myocardial ischaemia due to coronary disorders was alluded, but microscopic study of the transplant showed that plasma-cell invasion in perivascular region at early stage and localized necrosis of myocardium sporadically on the third day and especially perivascular change or Ashoff's Knoten were no indication of myocardial ischemia, but allergic reaction was strongly suggested by these findings quite as in transplantation of other organ. The present author tried to prolong the viability of the transplant by using corticoid in a case but failed in the trial. Anyhow heart transplantation may be useful for study of common troubles in transplantation, because the pulsating transplant does not necessitate biopsies.

SUMMARY

In order to find an approved technique and to study the general results of homologous canine heart transplantation, twenty-nine experiments were performed in three groups of dogs. After evaluations of the experiments with ECG, and pathological examination, the following results were obtained:

1. Twenty-nine transplantations were attempted and all were technically successful but one failed by coronary air-embolism.

2. Twelve transplants failed within 10 hours and most of the first group (5 of 6 cases) failed by left atrial rupture.

3. The third group, subjected to end-to-side anastomosis between the aorta and the carotid artery, showed most satisfactory results.

4. The longest period of survival of the transplant was 130 hours. ECG or pathological study was made with the transplant, and incompatibility of the transplant was discussed by pathological findings.

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Experimental Studies on Homologous Heart Transplantation

Second Report: Pharmacological Studies on the Transplanted Heart

By

Fumio Jin

From the Surgical Clinic of Prof. S.T. Katsura, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, November 25, 1959)

Though isolated heart preparations have been widely used for study of physiology and pharmacology of the heart, the heart transplantation was suggested as a new device for such purposes by Mann and his associates[1] in 1933. They studied the pharmacological effect of thyroxin on the transplanted heart by using the cervical canine heart transplantation which was a first invention in this field. Since their excellent report, there is almost no communication in the literature except a study of Marcus *et al.*[2]

As a technical device for study of the heart, the heart tansplantation would likely have some advantages over the isolated heart preparation; the denervated heart's functions are easily observed in comparison with the activity of normal heart in the same animal's body.

With such a fact in mind, the author of this paper undertook the following experiments, for the purpose of studying the behavior of the denervated heart in comparison with the normal heart in the same animal's body against various kinds of drugs.

METHOD

Mongrel dogs were used for the experiments and the heart was transplanted in the same manner as described in Report I. After comfirming that the heart rate of the transplant was stable by ECG record following endotracheal ether anesthesia induced in the recipient dog, the below mentioned drugs were injected intravenously from the leg. All drugs were diluted with 5% glucose solution to the volume of 5 cc and injected at rate of 2.0 cc per minute. Following the injection, the V-lead of ECG was taken at 30 seconds, intervals for the first 5 minutes, at 2 minutes' intervals thereafter and the cardiac rate of the transplant and the reciplient's heart were recorded.

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The drugs employed in this experiments were as follows.

1. Vasopressants

Epinephrine : 0.0034 and 0.028 mg. per kg. of the recipient's weight.
Nor-adrenaline : 0.0055 and 0.004 mg. per kg.
Neosynephrine : 0.022 and 0.01 mg. per kg.
Ephedrine : 2.3 and 1.5 mg. per kg.

2. Central nerve stimulants

Cardiazol (Pentamethylenetetrazol) : 0.015 mg. and 0.01 mg. per kg.
Aminocordine (Coramine) : 18 mg. per kg.

3. Drugs acting on the parasympathetic nervous system

Atropine : 0.038 and 0.05 mg. per kg.
Pilocarpine : 0.6 and 0.3 mg. per kg.

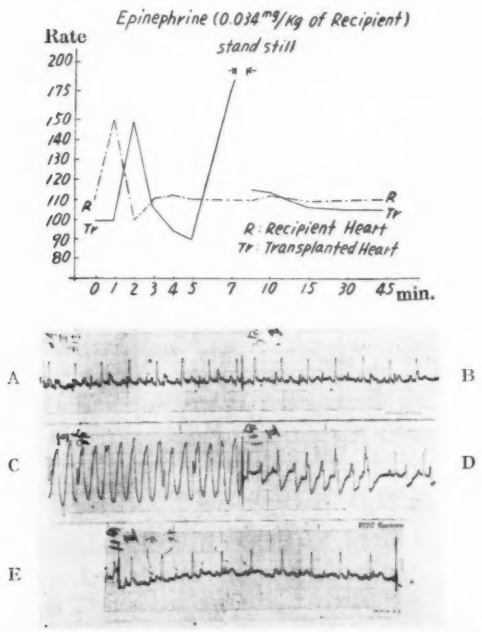

Fig. 1. The ECG of V-lead was traced simultaneously with the above graph of transplanted cardiac rates. A : 2 min. after injection. B : 3 min. after injection. C : Ventricular fibrillation of transplanted heart at 7 min. after injection. D : Transplanted heart starts again to beat after systolic cardiac standstill. E : ECG of transplant at 11 min. after injection is nearly the same as before injection. The small deflections of ECG are the transplant's and the large complexes are the recipient's.

RESULTS

Vasopressants: Epinephrine especially showed a distinguished pattern in its action on the rate of both hearts. Immediately following the injection the rate became rapid in one to two minutes, then a counteractive bradycardia developed and again the cardiac rates were increased. To a large amount of the drug, the transplanted heart reacted more intensively than the recipient heart and finally a systolic cardiac standstill ensued from ventricular tachycardia of the transplanted heart (Figs. 1 & 2 A).

Nor-adrenaline coused tachycardia in both the hearts for a period of one hour. The transplanted heart sometimes showed temporarily slow cardiac rates two to four minutes after the injection (Fig. 2 B).

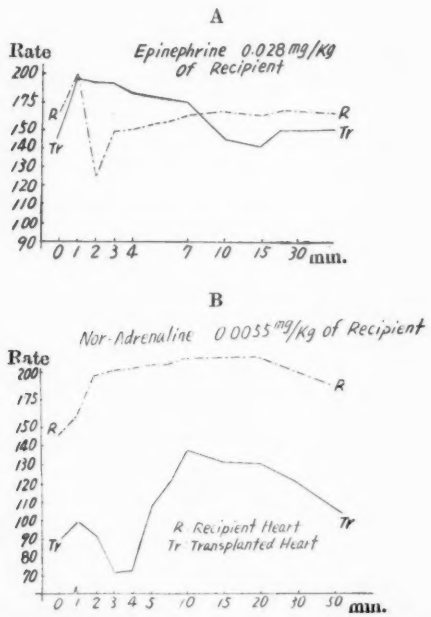

Fig. 2. The upper graph A shows hypersensitivity of transplant to relative small amount of epinephrine. The lower graph B shows the accelerating effect of nor-adrenaline.

In the group given neosynephrine, the cardiac rate in both the hearts was reduced to about 10 to 20 per minute without exception (Fig. 3 A).

Ephedrine acted as an accelerator on both the hearts but its action on the transplant was stronger than on the recipient heart (Fig. 3 B).

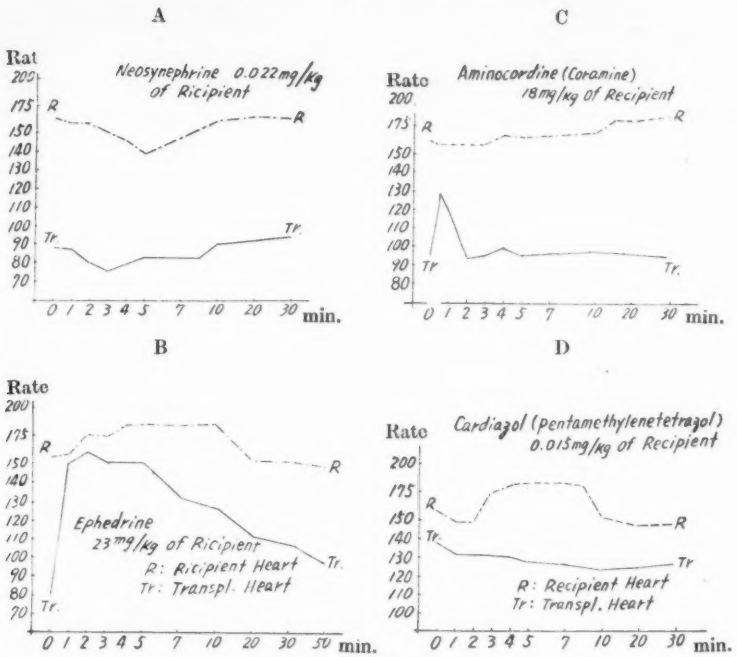

Fig. 3. A : neosynephrine causes bradycardia in both recipient and transplanted hearts. B : transplanted heart showed hypersensitivity to ephedrine. C : the initial tachycardia by aminocordine in transplanted heart is probably due to lightening of anesthesia. D : cardiazol causes no remarkable effect on the rate of transplanted heart.

Central nerve stimulants : With both cardiazol and coramine, the cardiac rates were not changed remarkably (Fig. 3 A & B). Increased cardiac rates with the transplanted heart after coramine in Fig. 3 C was likely due to lightening of the anesthesia.

Parasympathetic drugs : Though a large amount of atropine induced slightly rapid cardiac rate, pilocarpine displayed an intense action on the recipient heart (Fig. 4 A). After injection of pilocarpine, the recipient heart rapidly slowed down in about 3 minutes and finally developed cardiac standstill, whereas the transplanted heart showed slightly slow cardiac rate even when the recipient heart was in cardiac standstill (Fig. 4 B).

DISCUSSION

From these experiments, it is found that the heart transplantation is a very good method for study of the physiology or pharmacology of the denervated,

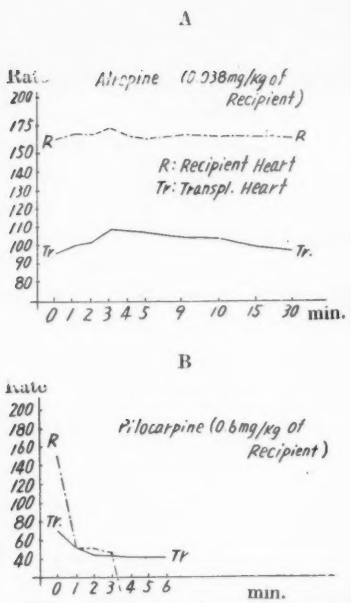

Fig. 4. The upper groph shows the effect of atropine : slight tachycardia in both hearts. The lower shows the effect of pilocarpine : recipient heart showed cardiac standstill after intensified bradycardia at 3 min. while transplanted heart is keeping a proper beat.

namely, transplanted heart in comparison with the normal recipient heart's action in the same body. Pharmacological study of the transplanted heart has interested only few investigators and we find only two communications : Mann and his associates reported on thyroxin and Marcus and his coworkers studied on digitalis or epinephrine without detailed protocol. For many years it has been known that certain organs became more sensitive to drugs when deprived of their nerve supply.[3),4)] With such previous reports in my mind, the problem of the supersensitivity of the denervated heart was studied. With epinephrine the denervated heart showed a very intense reaction in comparison with the recipient heart and in large dosis the transplanted heart suffered a systolic cardiac standstill, whereas the recipient heart showed a regular biphasic epinephrine reaction. In this experiment, the sensitivity of denervated heart is high in the order of epinephrine, ephedrine and nor-adrenaline. It is interesting that nor-adrenaline shows slight tachycardia in both the hearts,whereas bradycardia is usually induced by its clinical application. Bradycardia following neosynephrine injection is said to be due to vagal stimulation[5)], but in this experiment the drug showed some

direct myocardial action so far as bradycardia of the denervated heart is concerned. Before making the tests with central nerve stimulants the author expected something from its actions because the transplanted heart easily inclined to a tachycardia following a light exercise or excitement of the recipient dog. Neither aminocordine nor cardiazol showed any perceptible effect on the hearts. Sympathomimetic drugs mostly induced sensitivity of the denervated heart, but to parasympathetic drugs the denervated heart showed strong resistance. These contradictory facts on the sensitivity of the denervated heart may need further study in future.

SUMMARY

The cervical heart transplantation is a new and promising device for study of pharmacology of the denervated heart. In this experiments, pharmacological studies of vasopressants, central nervous stimulants and parasympathetic drugs on the denervated heart are studied in comparison with the recipient heart in the same body.

1. As a technique for investigating the denervated heart, the usefulness of the cervical heart transplantation described by Mann, Priestley, Marcowitz and Yater was proved.

2. Highsensitivity of the denervated heart to sympathomimetic drugs was found to be in the order of epinephrine, ephedrine and nor-adrenaline.

3. On the contrary, parasympathetics evoked no highsensitivity of the denervated heart.

4. Central nerve stimulants have no action on the denervated heart.

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Experimental Studies on Homologous Heart Transplantation

Third Report : The Limits of Restoration of Preserved Heart

By

Fumio Jin

From the Surgical Clinic of Prof. S.T. Katsura, Faculty of Medicine, Tohoku University, Sendai

(Received for publication, November 25, 1959)

The tolerance limits of the heart to acute ischemia has been regarded as relatively short due to its metabolic demands. The author however feels that the heart may stand against acute ischemia for a somewhat longer period, through the previous experimental study of homologous canine heart transplantation. In this study, even though the heart was excluded from the circulation for periods of 45 to 90 minuts, 28 among 29 hearts survived with normal cardiac rhythm.[1)]

Crowell and his associates[2)] have reported that innumerable microclots were produced by the acids of metabolic products in the blood vessels within 3 to 5 minutes of circulatory cessation. Prevention of this blood coagulation by heparin as an anti-coagulant may prolong the period of restoration, and clinical experience recently has shown that heparinization produces tolerance to a longer period of total circulatory arrest. The other principle to prolong the restoration period of the heart excluded from circulation, may be hypothermia. With the aim of reducing metabolism of tissue, hypothermia has been widely used experimentally as well as clinically.

The present communication was designed to determine whether a preserved isolated heart can survive for prolonged periods and what conditions can make such heart revive with normal cardiac rhythm.

METHOD

Thirty eight of mongrel puppies weighing 1.0 to 2.7 kg. were used. The puppy's hearts were transplanted to the neck of adult dogs in the same manner as stated in the previous paper[1)]. The experimental animals were divided into three groups.

Group 1. Under open drop ether anesthesia, midsternal splitting incision

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was made on the puppy and the heart was extracted without heparin as described previously. The isolated hearts were preserved in Ringer's, Tyrode's, 5% glucose or 0.9% NaCl solution at various temperatures for periods of 2 to 3, 5, 6, and up to 25 hours. Following the cervical heart transplantation, the cardiac tone and rhythm were observed in these hearts.

Group 2. Methods of the operations were the same as in Group 1, but the pupp'ys heart was completely heparinized when it was excised.

Group 3. After removing the heart with the lungs, the solutions were perfused through the right atrium and allowed to empty through a small incision made in the descending aorta proximal to the ligature. During the perfusion, care must be taken to avoid the dilatation of the right atrium. After completion of the wash out of the heart and lungs, the descending aorta was again ligated more proximal to the incision, and the lungs were removed from the heart. The methods of the preservation were just the same as in Group 1.

RESULT

Group 1. Three hearts preserved for periods of two to three hours at 19°C survived with good cardiac tone and normal cardiac rhythm, but when preserved for 5 to 6 hours at 25°C they failed to recover the activity. Three hearts preserved for 24 hours period at 18° to 5°C could not return to a normal rhythm (Table 1).

TABLE I. Restoration of Function of the Hypothermically Preserved Heart without Heparinization or Perfusion

Hours	Nos. of exp.	Temperature (°C)	Preserving sol.	Result
2–3	2	19	Ringer's	Good
2	1	19	Tyrode's	Good
5–6	2	19	Ringer's	Fail
5–6	2	25	Ringer's	Fail
24–25	2	18	Ringer's	Fail
24	1	5	Ringer's	Fail

Group 2 (heparinized heart series). A heart preserved at 4°C for 30 hours period was successfully resuscitated to normal rhythm. After preservation of 24 to 25 hours at 5 to 4°C, 4 cases were able to recover a regular rhythm, whereas a case at 24°C did not show a cardiac rhythm. 48 hours preservation prevented resuscitation of the cardiac function (Table 2).

Group 3. (perfused heart series). Eight hearts preserved for 20 to 24 hours in Tyrode's or Ringer's solution at 5°C were able to recover good heart contraction with normal rhythm, whereas 2 cases with 5% glucose or 0.9% Nacl started in atrial contraction but never returned to normal cardiac rhythm. At the temperature of 25°C, two hearts never revived with normal heart contrac-

TABLE II. Restoration of Function of the Hypothermic Heart with Heparinization (10 mg.)

Hours	Nos. of exp.	Temperature (C)	Immersed sol.	Result
5–6	2	20	Ringer's	Good
5–6	1	4	Ringer's	Excellent
5–6	1	4	5% Glucose	Excellent
24–25	1	24	Ringer's	Fail
24–25	3	5	Ringer's	Good (2)
24	1	4	5% Glucose	Good
30	1	4	5% Glucose	Good
48	1	4	5% Glucose	Fail

TABLE III. Restoration of Function of the Hypothermic Heart with Complete Perfusion

Hours	Nos. of exp.	Temperature (C)	Perfused sol.	Result
6–8	2	5	Ringer's	Good
20–24	3	5	Tyrode's	Good (2)
20–24	5	5	Ringer's	Good (3)
24	1	25	Ringer's	Fail
24	1	25	Tyrode's	Fail
24	1	5	5% Glucose	only atrial response
24	1	5	0.9% Nacl	same as above
30	1	4	Tyrode's	Fair
48–50	2	4	Tyrode's	Fail

tions. After 30 hours' preservation the heart started with fine fibrillation and an electric counter shock succeeded in restoring a normal cardiac rhythm. Two hearts preserved for 48 hours period were never back to adequate cardiac contraction.

DISCUSSION

From these results, the limits of restoration of the preserved heart are at least in relation to the presevation of patency of the capillary bed and does not depend solely on the length of circulatory occlusion. In the author's previous experiments, hearts were surprisingly restored the cardiac rhythm following long circulatory cessation as much as 45 to 90 minutes and the author was expecting that heart might be as strong as a muscle against acute ischemia under certain conditions.

There have been not so many reports on the reviving limits of a heart against total circulatory cessation, though a lot of investigations have been reported on the biochemical study of myocardial slices but these are not a whole organ having a cardiac function. In 1936 Kuntz[3] reported that adult human hearts at autopsy were almost always restored to cardiac contraction within 30 minutes after death and Ossinowsky[1] stated that children's hearts revived up to 28 hours after death but there were no detailed protocol whether the contractions appeared

in a normal rhythm or not. These previous reports strongly suggest a possibility of some extension of the myocardiac viability with circulatory cessation. While this study was proceeding, we have found a couple reports of Howard and Webb[5] in which the myocardial viability was investigated by heart transplantation as the present author did in this paper and they concluded that the time limits of viability of the unoxygenated myocardium are related to the preservation of patent capillary bed and an excised heart may be presumed the function up to 90 minutes at room temperature. They emphasized also that perfusion may prolong the restoration limits of the heart and perfused heart can be preserved for periods of 6 to 8 hours at 4°C. Compared with their conclusion, the present author's results show that heparinization makes it possible to prolong the time limits for the preserved heart but there is a great descrepancy in a survival limits. These different results may be due to differences in the experimental techniques, namely, the practice of the heart transplantation, the amount of heparin, the perfusion and the kind of the preserving solutions, even though the principle of prolongation of the preserved heart is quite the same in both experiments. Recently, Matsuda and his associates[6] have claimed that the myocardial membrane potential of canine ventricular wall completely resumed its resting and action potentials after 24 to 48 hours preservation at 1° to 3°C. Their experimental results would seem to support the author's result from stand point of electrophysiological view.

SUMMARY

The limits of myocardial tolerance of the preserved canine heart at various conditions were studied by the heart transplantation method.

1. Without heparinization of the heart, the time limit may be 2 to 3 hours at 19°C.

2. Preventing blood coagulation by heavy heparinization, preserved hearts may be shut out of circulation up to 30 hours and resume a normal cardiac rhythm.

3. Perfusion of the coronary vessels to wash out all blood extends the limits as does complete heparinization.

4. The time limits of viability of preserved heart are related not solely to the length of acute ischemia but more to the patency of capillary bed. Hypothermic preservation is another very effective method to extend the limits, whereas the kind of preserving solutions showed no conclusive difference in the present experiments.

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Scabies Norvegica Associated with Leukemia Cutis

(Chronic Myeloid Leukemia)

By

Tadashi Tanaka and **Makoto Usuba**

Department of Dermatology, Tohoku University School of Medicine, Sendai; Derector: Prof. Y. Takahashi

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INTRODUCTION

Scabies norvegica, which was first described by Boeck, has been elucidated in its etiology by the detailed discussions of Boeck and Danielssen. Since then about 120 cases were reported in Japan and Western countries.

The authors have recently experienced a case of this disease associated with leukemia cutis (chronic myeloid leukemia), as reported in the following.

Report of a Case

A Japanese female, wife of a fisher, 49 years of age.

1. History

The patient had been healthy since infancy and experienced no mentionable disease.

Small papules began to appear and to be disseminated from the elbow pits and forearms speedily to the trunk about 13 years ago, accompanying severe itching. They nearly disappeared within a year without receiving a mentionable medicinal treatment, but thereafter similar papules reappeared now and then in the genitocrural parts, the cubital and popliteal folds. In those days, about half of her 12 family members were said to have been suffering from typical scabies.

About 7 years ago, skin lesions with considerably thickened scales broke out on the extensor side of the forearms and when such lesions began to spread over the limbs, trunk and head, the patient consulted physicians at several hospitals and received various treatments, but though temporary relief was achieved, similar lesions appeared again in two months or so.

Since then, the eruption persisted with occasional remissions and remaining extremely recalcitrant to any treatment, the patient was admitted to the Dermatology Department of the Tohoku University Hospital in January, 1957.

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2. Clinical Picture and Laboratory Findings at the Time of Hospitalization

The patient was well developed physically but showed a tendency to inanition, and anemia was apparent in the conjunctivas of the eyelids.

The results of simple mental tests showed that her intelligence was of the level of moronity.

The skin surface was generally pigmented dark gray-brown, was dry and dirty.

The most noteworthy feature was the peculiar scales on the skin lesions, which were disseminated on the trunk, particularly densely on the ventral side and less on the dorsal side. The individual lesions were covered with irregularly shaped, dry, gray-white or dirty gray-brown scales, markedly thickend in plate-like, pulvinate or sometimes umbonate form, to the size of a finger-tip or a coin, and sometimes even larger. The lesions were, for the most part, raised up to 2–3 mm., but rarely up to 5–6 mm. Their surface was mostly uneven and irregular, owing to the presence of complex furrows variable in depth and of small papillary upheavals. They were surrounded by similarly formed but smaller satellites or by fine gray-white scale dusts adhering to the skin surface diffusely and densely. The scales adhered firmly to the under surface and could not be detached with ease, and if they were forcibly scraped off, they characteristically came off in condensed furfuraceous masses but never in lamellae as in psoriasis. The exposed under surface usually showed a little serous exudation or a uniform bleeding. A few similar rupioid lesions were found on the labia majora and the perianal part, too.

While lesions were densely packed and coalesced on the extensor side of the joints of the limbs, their number was unexpectedly very small in the cubital and popliteal folds, the axillae and the inguinal regions. In particular, whereas scales were found on the extensor side of all the joints of the phalanges without exception, between the fingers only very thin, if any, desquamation was observed.

The nails were glossy as if carefully manicured, as is often seen in itchy skin diseases (the patient, however, denied any itchy sensation in her eruption). The nail surface was severely rugged owing to presence of longitudinal ridges, grooves and pits, but no opacity, hypertrophy, fragility of the nails and paronychial changes were observable.

Except a considerable thickneing of the skin surface, no scales were found at all on the palms and soles, but the patient stated that in the past she found scales in these parts.

On the face a few lesions were sporadically found in the superciliary regions, but on the ear-lobes and nostrils diffuse scales were adhered.

On the scalp depilation was marked in some parts of the occipital and temporal regions. The rupioid scales in these parts were riddled with pits enclosing bundles of few hairs growing out of the hair follicles.

Fig. 1.

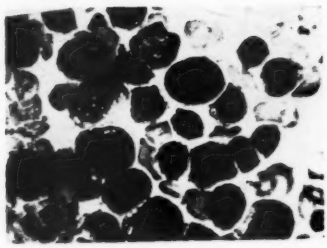

Fig. 2.

Fig. 1. Disseminated rupia-like lesions.
Fig. 2. Polymorphous immature myeloid leucocytes in peripheral blood, reminding of marrow picture.

TABLE I. Peripheral Blood Picture

Kinds of Leucocytes	%	Kinds of Leucocytes	%
Myeloblasts	70.5	Staff cells	4.5
Promyelocytes	4.5	Lobocytes	9.5
Myelocytes	5.0	II	2.5
Metamyelocytes	2.0	III	6.5
Eosinophilic cells	0	IV	0.5
Basophilic cells	0	V	0
Plasma cells	0	Lymphocytes	4.0

At our first examination, a gigantic splenic tumor nearly reaching beneath the umbilicus was palpable. (The patient said to have been conscious of this since 6 months.) The abdomen was distended (girth : 86 cm.), showing fluctuation but no abnormalities in E.C.G. At the same time, the leucocyte count of 102,400 per cubic millimeter was obtained in the peripheral blood and by closer examinations of the bone marrow smears, complication of chronic myeloid leukemia was confirmed (Fig. 2 and Tab. I),

The results of laboratory tests at the time of hospitalization are summarized as follows :

Tuberculin test, 0×0 mm.; blood pressure, 108–52 mm.Hg.; basal metabolic rate, +33% ; serum Takada reaction, 2 + ; thymol turbidity test, 4.2 ; urobilinogen in urine, 1 + ; sedimentation rate, 7 mm. in first hour and 11 mm. in second hour ; serum Wassermann reaction, negative; total protein in plasma, 7.41 gm. /dl. (albumin, 3.26 gm. ; globulin 4.15 gm. ; A/G, 0.78) ; γ-globulin, 31.9% ; total cholesterol in serum, 182.1 mg. /dl.

3. Course after Hospitalization

Leukemia was treated with a nitrogen mustard N-oxide preparation with good results and the splenic tumor was reduced by one half in bulk in 20 days after its first injection. This compound, however, had no effect upon the skin lesions.

Soon after hospitalization, typical mites of human scabies, Sarcoptes scabiei var. hominis, were demonstrated in the scales and the diagnosis of scabies norvegica was confirmed. Since then powder and ointment of γ-BHC and DDT were used in combination and the skin lesions improved, leaving more or less dark brown pigmented spots.

In the interim, pinhead-sized vesicles and papules broke out epidemically between the fingers, on the forearms and chest at first of the patient's husband and some inmates of the same ward, then of the attendant women and nurses, but repeated examinations failed in showing existence of any mite in these subjects.

About 3 weeks before the patient died, millet- to small bean-sized solid papules appeared on the sternum, epigastrium and chest, partly in groups and partly in coalescence. They showed flat, hemispheric elevations, were like the normal skin in color and somewhat lustrous, and those developing in number on the cheeks and chin were colored lightred. Thus, a case of papular leukemia cutis was present here.

Later on, as the liver grew swollen, large and small purpura appeared on the face and trunk and severe jaundice set in. The patient's appetite was totally suppressed, and blood transfusion and parenteral fluid supply were applied every day, while cortisone was administered, seeing that the result of Thorn's test registered –27.7%, but no improvement could be brought in any of her conditions. Inanition went on quickly, and she died on June 27, 1957. The results of laboratory tests preceding her death are summarized as follows:

Sedimantation rate, 2 mm. in first hour and 4 mm. in second hour; blood coagulation time, 160 min.; γ-globulin in serum, 26.3%; leucocyte count, 284, 600; B.M.R. + 33.2%. Eosinophils in peripheral blood decreased from 12% at the time of the first hospitalization gradually to 1%– 0 and remained at this level for some time before her death.

Detection rate of male and female mites in the scales is shown in Tab. II.

4. Histological Findings

Rupioid Lesion (Fig. 3).

The remarkably thickend horny layer contained burrows forming many layers

Table II. Male-Female Ratio of Mites Detected

Male-Female Ratio of Acarus Found in Scales			Distribution Among 300 Mites in Total		
Author	♀	♂	Acarus	Number	%
Leydig	100	2	♂	13	4.3
Bergh	100	40.0	♀	53	17.7
Iziri	100	24	Larvae	94	31.3
Iziri	100	23	Ova	140	46.7
Hirota	100	23.1			
Hirota	100	40.7			
Tanaka & Usuba	100	24.5			

of large and small complexly partitioned apartments, the beams of which were formed by layers of parakeratotic cells and the inner walls of the burrows were almost entirely lined with a thin acidophilic layer.

The mites showed varied cross-sections cheifly in the deeper part of the rupioid layer, and the majority of them wore a thin, rather light-staining acidophilic layer as their outer shell.

The granular layer was partly absent. Direct transition from the thin acidophilic and parakeratotic layer forming the septa between the burrows into the

Fig. 3. A section of rupioid scabies lesion. 60×. H.-E. stain. Remarkable thickening of the horny layer. Many-layered burrows and multiform cross-sections of mites in them. Acanthosis and papillomatosis.

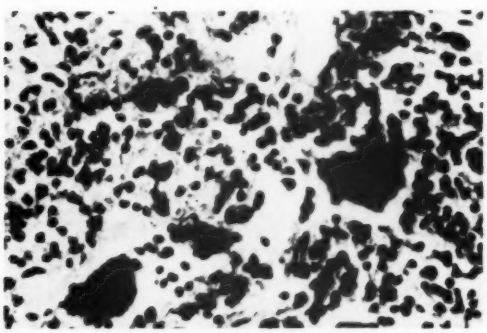

Fig. 4. Histological section of papular leukemid on the breast. Densely infiltrated portion beneath the papillary layer. Myeloid cells with clear nuclei. Lymphatic cells of smaller size with dark-staining nuclei. Several giant cells. A small number of histiocytes with nuclei having a coarse chromatin network.

prickle-cell layer was observed. The obvious elongation and thickening of the prickel-cell layer and the corresponding elongation of the papillae were found. In the papillary and the subpapillary layers, there were edema and cell infiltration of lymphocytes in the main, but containing no eosinophilic cells.

Papule of Leukemia Cutis (Fig. 4).

Specimens were taken from the lesions on the chest. The infiltrations forming the papular eruption mainly consisted of following 4 cell types:

1) Comparatively small cells with somewhat pale-staining nuclei, which were recognized as myelocytes.

2) Smaller cells with very dark-staining nuclei, namely lymphocytes.

3) Not a few sporadic giant cells containing more than a dozen pale-staining nuclei. The nuclei resembled closely those in the myelocytes mentioned in 1) above, but the cytoplasm was basophilic, unlike the acidophilic cytoplasm of giant cells proper.

The nature of such giant cells with basophilic cytoplasm has never been described, as far as we are aware, and it is impossible for us to assign them to any type of giant cells, but it is certain that they are anything but foreign-body giant cells.

4) Cells with oval or kidney-shaped nuclei, having a rather coarse chromatin network, i.e. histiocytes.

Excepting the giant cells, the above elements were found first to aggregate forming perivascular infiltration in the deeper corium, then to ascend obliquely through the reticular layer in thick cords or palisade-like formation, reaching thus the subpapillary layer, and to spread out there to form diffuse but conspicuously dense infiltration zone occupying nearly the whole thickness of the papillary layer.

DISCUSSION

The only one case of scabies norvegica associated with leukemia has been the case (55 years, ♀) reported by Dostrovsky[1] (1956) with a clinical picture of erythroderma presumably caused jointly by these two diseases, and lymphatic leukemic cell infiltration and acarus were demonstrated by histological examination. Our case is thus the second one of similar complication reported in the world.

Concerning epidemic infection of scabies norvegica, Hirota[2] asserted that familial infection was rare and the reports on the subject in Japan[3),4)] have been in agreement therewith, while Pautrier[5] recorded epidemic infection to 16 cases and Ingram[6] to 37 cases including a physician and a nurse. In the epidemic affecting more then 20 subjects, recorded by Dostrovsky, the fuilure in proving mites in any single case is in agreement with our findings. Vanni[7] also reported on similar experience. Tas[8] stated that in such infected cases of scabies, the

burrows were often absent, only small papules with severe itching being formed throughout, and Cernea[9] found some cases free of mites among the numerous cases suspected of scabies, during the epidemical prevalence of the disease after the World War II.

SUMMARY

The authors presented a report on a case of scabies norvegica associated with chronic myeloid leukemia accompanied with skin lesions.

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Histological and Histochemical Observations on the Developing Pancreas of Fetal Mouse

By

Tom Mori and **Akinobu Haga**

From the Department of Anatomy, Tohoku University School of Medicine, Sendai

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In most of the previous studies concerning the ontogenesis of the pancreas, the special attention of the investigators has been centered on the development of the islets of Langerhans, since in the pioneer works of Laguesse[1)2)] two successive stages in the development of the islets, îlots primaires and îlots secondaires, were described. According to Laguesse's opinion, the îlots primaires are derived from the primitive cell cord or the primitive tubules of the dorsal pancreatic anlage and form large masses of the islet cells, the îlots composées. Then, they undergo degeneration accompanying the formation of the excretory acini, from which the îlots secondaires differentiate through the de-differentiation of the acinar cells. Furthermore, Laguesse stated that such an acino-insular transformation may even take place in an opposite direction (théorie de balancement).

These two steps in the development of pancreatic islets were confirmed later by many researchers, some of them calling the primordia of the islets corresponding to the primary islets by special names such as "Inselanlagen" (Siwe[3)]) or "primäre grosse Inseln" (Yasunaka[1)]). But the "théorie de blancement" was challenged by most of the investigators though some confirmed the acinar origin of the secondary islets such as Neubert[5)], Freise[6)], Ferner and Stoeckenius[7)]. Recently, Bencosme[8)] also observed the formation of the islet from the undifferentiated primitive islet cells intercalated in the glandular epithelium of the excretory part, but he emphasized that there was no evidence of direct transformation of the fully developed acinar cells into the islet cells. This point of the problem under dispute dealing with the origin of the islet has been reviewed by Siwe[3)] and Bargmann[9)].

The primitive islet cells belonging to both the primary and the secondary islets were usually described by most of the authors as the cells stained deeply with eosin and containing pale stained vesicular nucleus. Some of them nominated these cells by such special names as "cellule trouble" (Laguesse), "trübe Zellen" (Neubert), or "muddy cells" (Warren and LeCompte[10)]), according to

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their characteristic appearance in the sections.

Regarding the histochemical properties of these developing islet cells, McAlpine[11] pointed out an intense activity of alkaline phosphatase in fetal and neonatal rats, and Hultquist and Thorell[12] described a high content of ribonucleic acid (RNA) in the primitive islet cells arranged in the wall of the pancreatic duct of human fetal specimens.

In this work, chronological alterations during the differentiation in some histochemical properties of the exocrine and the endocrine cells of the mouse pancreas are observed. During the course of the study, although the histochemical characteristics were mainly treated, a general observation of the histogenetical process of the pancreas was also made under an attempt to give some contribution to the problem dealing with the origin of the pancreatic islets.

MATERIAL AND METHODS

Fetus of dd-strain mouse sampled at the half day-intervals from 9½ to 19½ days of pregnancy were used. The latter time period is just before the birth in this strain of mouse. The ages of the fetus were computed from the observed time of successful copulation, the same hour in the next day being dated as the end of the first day of pregnancy.

The specimens for the detection of alkaline and acid phosphatases and lipase were fixed and dehydrated in chilled acetone, while those for determining the ribonucleic acid (RNA) were fixed in Carnoy's fluid. A part of the specimens was also fixed in Zenker-formol or in 10% formalin. All the specimens were imbedded in paraffin, and cut serially in transverse plane at 5–8 μ.

The detection of the enzyme activities was carried out according to the method of Gomori[13], using sodium beta-glycerophosphate, a mixed preparation of alpha- and beta-glycerophosphate and "Tween 60" as the substrates for alkaline phosphatase, acid phosphatase and lipase, respectively. The incubation was done without previous deparaffinization of the sections. The distribution of RNA was tested by staining the Carnoy-fixed sections with methyl green and pyronin, and partially with chrome alum gallocyanin of Einarson[14]. Specimens fixed in Zenker-formol were used for the general observations on the histogenesis after the staining with hematoxylin and eosin (H-E), except some sections stained with Altmann's anilline water-acid fuchsin and picric acid which yields a brilliantly red tint of the zymogen granules. Those fixed in formalin were stained with Gomori's[15] aldehyde-fuchsin (A-F) according to the slight modification of Mori, Fujimori and Kato[16] in order to detect the beta-cell-granules.

OBSERVATIONS

General description of the histogenesis and the distribution of RNA.

9½ to 10½ days: The dorsal anlage of the pancreas was initially found in the fetus of 9½ days of gestation as an epithelial cell cord containing a considerably wide evagination from the intestinal cavity in its proximal part. The ventral pancreatic anlage appeared on the 10th day as a well-defined evagination taking its origin from the proximal part of the hepatic diverticulum adjacent to the primitive duodenum. The cytoplasm of the cells of both the dorsal and the ventral anlagen was stained intensely with pyronin, especially in the cells situated in the peripheral and distal parts of the anlagen. In the dorsal pancreas

of 10 days, a small number of cells were stained with pyronin more intensely than the remaining cells of the anlage. These intensely basophilic cells were found intermingled with the epithelial cells, or in the small groups at the distal end of the anlage, suggesting the emigration and protrusion of these cells out from the deep part of the dorsal anlage (Fig. 1).

In the slightly advanced embryos dated 10½ days, the specific cells noticed in the 10-day-specimens with dominant cytoplasmic basophilia were found in larger groups attached at the posterior end of the dorsal anlage (Fig. 2). The basophilia was however markedly reduced in this stage, the majority of these cells revealing less affnity to pyronin than the cells in the main part of the dorsal pancreas. They were considerably large, and showed indistinct outline of the cell body. The nuclei were also large and palely stained with methyl green, since the chromatin particles contained were rather fine. In the sections stained with H-E., these specific cells were well defined by the eosinophilic cell body surrounding a vesicular nucleus from the darkly stained main cells of the anlage. The mass of these eosinophilic cells is undoubtedly just the primary islet described by many authors in man and in various animals, and the "primäre grosse Insel" described by Yasunaka[4)] in fetal mouse.

11 to 11½ days: The diverticulum in the dorsal pancreatic anlage almost disappeared leaving a soild cell cord, the primitive dorsal pancreatic duct, connecting the distal part of the anlage and the duodenum. The outline of the dorsal pancreas became uneven by slight bulging out of the cells in many parts of the anlage. The most superficial cells of the anlage took an appearance of simple columnar epithelium, the cytoplasm of which showed an intense affinity to pryonin. The masses of the primary islets, which stood out by diffuse and pale tint of the cytoplasm, were found in a local fusion with each other.

12 to 12½ days: The outline of the dorsal anlage became very irregular by prominent bulgings in many parts of the anlage. These bulges were separated from each other by deep indentations containing mesenchyme tissue and blood capillaries. This change sets to the ramification of the primary pancreatic tubules and the adenomere formation (Fig. 3). Corresponding to these ramifications and the formation of the primitive acini, the epithelial cells forming the surface of these bulges were stained intensely with pyronin, while the cells in the internal part of the dorsal pancreas were arranged less dense and showed rather limited cytoplasmic basophilia. The mass of the primary islets was stained diffusely and faintly with pyronin, and the various parts of the islets were found scattered between the bulgings of the anlage, sometimes being in touch with them. In the dorsal pancreas of this stage, especially after 12½ days, a new formation of the islets took place from the walls of the tubules which now had minute tubular cavities. In the H-E-stained sections, these new islets were observed as small groups of cells stained diffusely and remarkably with eosin and were protruding

perpendicularly from the walls of the tubules (Fig. 3). The similar cells in other specimens showed only a limited affinity to pyronin, and to chrome alum gallocyanin, either. These new islets deriving from the duct system are just what were nominated as "primäre kleine Inseln" by Yasunaka[1], and we think that they are the seconary islets in the earliest stage.

13 to 13½ days: The formation of the excretory units became very distinct in the major parts of the dorsal pancreatic anlage. Some terminal buds of the acinus took a spherical form having a wide acinar cavity which was in communication with the tubular cavity. From the side wall of the duct, too, many primitive acini were projecting in spherical or semi-spherical bulges. The cells of these terminal and side buds stained very strongly with pyronin, especially the juxtanuclear area of their cytoplasm was loaded with a coarse basophilic substance. The nucleus usually contained two or three or more large and angular nucleoli which were also colored brilliantly red with pyronine. The cells of the duct system were less basophilic and their nucleoli were also smaller than those of the acinar cells in number and size. So, the boundaries of most acini to the adjacent duct cells were always clearly distinguished by an abrupt change in morphology of these two kinds of cells, especially by the marked difference in the intensity of cytoplasmic basophilia. The cells of the islets, including those of both the primary and the secondary ones, showed only a faint and diffuse tint after the staining with pyronin or gallocyanin.

From the walls of the fine primitive tubules of the ventral anlage of this stage, too, small groups of rather faintly stained cells were projecting. This was regarded as the first appearance of the secondary islets in the ventral pancreas, where, unlike in the dorsal one, no primary islet preceded the development of the secondary islet.

14 to 14½ days: The development of both the exocrine and the endocrine parts was greatly advanced. The cytoplasmic basophilia of the acinar cells became very conspicuous, and the perinuclear and the basal parts of most of the acinar cells were filled with numbers of coarse granules or thread-like substance which exhibited an intense affinity to pyronin.

The islets tended to unite with each other to form large band-like masses extending along, and partially connecting with, the duct. Yasunaka[1] discriminated this band-like formation of the islets from the preceding perpendicular projecting, and used the appelation of "sekundäre kleine Inseln" for these islets. The cells of these islets were also faintly stained with pyronin and gallocyanin (Fig. 4). As will be described below in the description concerning the phosphatase activity, the differentiation of the new islet cells from the duct wall is in progress throughout various parts of the pancreatic anlage, especially after 13 days and 6 hours. However, it was very difficult to point out each primitive islet cells arranged in the duct epithelia in the sections stained with methyl green and pyronin, or with

chrome alum gallocyanin, either in this or in the preceding stage. Only occasionally, single cells with intensely but diffusely basophilic cytoplasm were found intermingled among the cells of the duct epithelium, but we could not identify them with the primitive islet cells, since the number of these basophilic cells was far below that of the primitive islet cells standing out by their high phosphatase activity in the specimens of the same developmental stage.

15 to 15½ days: In the specimens of 15 days stained by Altmann's method, a few acinar cells were found containing minute granules stained with acid fuchsin, although the fuchsinophilia was yet indistinct and the number of the granules was very small. A half day later, this granules became larger in number and size, and the affinity to acid fuchsin was also highly enhanced. This may be identified as the zymogen granules.

In the dorsal pancreas, the composed mass of the islet was in a form of thick band extending along the duct or in a bridge-like cord of cells connecting the branches of the duct and occasionally acini. In a very limited number of these islet cells, fine beta-granules were stained faintly but distinctly with A-F.

16 to 19½ days: The dorsal and the ventral anlagen of the pancreas tended to join each other on the 16th day. After that, according to the rapid proliferation and enlargement of the excretory acini, the proportion of the interstitial connective tissue decreased. Most of the acinar cells came to contain many zymogen granules in the apical portion of their cell bodies before 17½ days, and the intensity of the cytoplasmic basophilia looked considerably reduced with the rapid appearance of the granules. The cavity of the proximal part of the dorsal pancreatic duct tended to disappear, and the basophilia of the epithelial cells in this portion of the duct was apparently less intense than in the other part of the duct. The beta granules showed a steady increase until they became apparent in a greater number of the islet cells in both the dorsal and the ventral parts of the pancreas on the 18th day. After the 18th day, the large masses of the islets underwent a separation into elliptic or spherical groups of cells, suggesting the formation of the definite Langerhans' islets.

Activities of alkaline and acid phosphatases and lipase.

In the following, the description is mainly centered on the distribution of alkaline phosphatase, since the activity of this enzyme is looked upon as to be more closely related to the differentiation of the islets than the other enzymes. As for acid phosphatase, only cursory accounts are presented because our results on the distribution of this enzyme often contained some irregularities.

9½ to 10½ days: At the initial stage of the formation, both the dorsal and the ventral anlagen of the pancreas revealed an intense activity of alkaline phosphatase, especially in the solid distal parts. This enzymic activity began to decrease in the subsequent stages, especially rapidly in the dorsal anlage, while

in the ventral one such a decline in the enzymic activity progressed more slowly leaving a perceptible black tint until the 12th day. In the dorsal anlage of 10 days, a small number of cells showing a high activity of alkaline phosphatase were found intermingled with the faintly stained cells, and some of these strongly reactive cells were in small groups attached to the distal end of the anlage (Fig. 5). A half day later, these cells of high phosphatase activity constituted a few large masses protruding from the distal part of the dorsal pancreas. This mass is undoubtedly the primary islet described above. A distinct activity of the enzyme was also observed in the proximal opening of the ventral pancreatic duct to the choledochus of 10½ days but this reaction disappeared in the succeeding stage.

The activity of acid phosphatase also looked slightly higher in the primary islets than in the main part of the dorsal pancreas of 10½ days.

11 to 11½ days: Along with the adenomere formation and the ramification of the tubules, the superficial cells of the dorsal pancreas showed a slight alkaline phosphatase activity corresponding to the bulging. As for the acid phosphatase, only moderate and diffuse reaction was observed throughout the anlagen, no noticeable difference being perceived between the cells of the primary islets and those of the main part of the anlage.

12 to 12½ days: As illustrated in Fig. 6, the outline of the dorsal anlage to the surrounding mesenchyme was not clearly defined, since the enzymic activities shown in both the tissues were almot on the same level of intensity. But the distal part of the dorsal anlage showed a distinct reactivity, especially in the primary islet and the epithelial bulges. A small number of highly stained cells were also observed inside the dorsal pancreas but these cells were not yet identified with the primitive cells of the secondary islet with certainty.

13 to 13½ days: With the rapid progress of the formation of the excretory acini and the general expansion of the anlage, the activity of alkaline phsophatase in the primitive acinar cells was considerably ehnanced (Fig. 7), but this reactivity diminished in the subsequent stage. In a specimen of 13 days and 6 hours, as shown in Fig. 7, we could point out intensely and definitly reactive cells intercalated in the barely stained duct epithelium, while in the 13-day-fetus, only a faint and doubtful reaction was found in a very limited number of cells arranged in the walls of the tubules. Later on, these highly stainable primitive cells of the secondary islet increased in number, and were most frequently found in the small branches of the duct including the terminal portion of the tubules adjacent to the acini. The primary islet still retained a slight activity of alkaline phosphatase.

The activity of acid phsophatase was moderate and rather diffuse, but in some specimens of 13½ days, a distinct reaction was found fairly restricted to the duct and the developing islets.

14 to 15 days: The primitive cells of the secondary islets showing a high activity of alkaline phosphatase were found most frequently in this stage. They were observed either as single cells arranged in the tubular epithelia or as small groups protruding laterally from the tubules (Figs. 8 and 9). Some of the centro-acinar cells also revealed an intense activity of this enzyme, though only on very rare occasions (Fig. 10). In this stage, such an intense enzymic activity was fairly restricted to the primitive islet cells, while the band-like mass of the islets showed only a faint or almost imperceptible tint.

The acid phosphatase reaction was observed mainly in the duct system and the islets, but it was not so clearly restricted to the latter as in the case of the alkaline phosphatase.

After 15 days, a minute amount of granular precipitates of pale-brown color could be observed in some of the acinar cells after the treatment for detecting the lipase activity.

15½ to 17 days: The intra-tubular primitive islet cells rapidly decreased in number unitl they had entirely disappeared by the 16th day. The islets went on growing and uniting with each other to form the larger complex masses, and the cells of these masses of the islets tended to resume a slight activity of alkaline phosphatase. In the specimens ranging from 15½ days to 16½ days, many cut surfaces of the islets were often standing out by their faint but distinct black tint against the other constitutents of the pancreas. The walls of the duct remained almost colorless, and some of the acinar cells showed very slight and granular reaction in the apical portion of the cell body.

After the treatment for detecting the acid phosphatase activity, very intense reaction of the duct and the islest was obtained, and the most of the acinar cells showed also a moderate reaction, especially in the nucleus (Fig. 12).

In the specimens of 16½ days, the precipitates representing the activity of lipase became rather coarse and their brown tint looked deeper than in the foregoing stage, but the amount was still small.

17½ to 19½ days: In this stage, the activity of alkaline phsophatase in the islets gradually diminished until a faint activity was left in the nuclei. But in the specimens of 19 days and 19½ days, a small number of cells located in the peripheral area of each definite islet often showed a moderate reaction in their cytoplasm and nucleus, this finding being in good agreement with that of McAlpine[11].

The activity of acid phosphatase suffered little reduction in the islets while that in the acinar cells almost diminished away after the 18th day (Fig. 13).

The activity of lipase was gradually intensified in this stage and most of the acinar cells came to contain coarse granular precipitates after 17½ days. The color of the precipitate also became deeper and deeper. The cells of the duct wall and the islets remained entirely negative to the lipase reaction.

DISCUSSION

Brachet[17)18)19)20)] and Caspersson[21)22)] greatly advanced our knowledge on the localization and the function of nucleic acids inside the cell using excellent technics which they devised, and the pancreas was one of the organs used in their early works on this subject (Brachet[17)18)] and Caspersson, Landström-Hydén and Aquilonius[23)]). Since then, thanks also to repeated examinations by numbers of researchers, it is nowadays widely known that the so-called ergastoplasm of the pancreatic acinar cells contain a large amount of RNA, while the islet cells almost lack in RNA (Vendrely and Vendrely[24)]).

As for the other histochemical characteristics of the pancreas, Gomori[25)] pointed out that the pancreas of mouse entirely lacked in the activity of alkaline phosphatase except in the capillary walls and in some parts of the duct system, and a selective localization of acid phosphatase in the islets was described by Noback and Montagna[26)]. Our findings in the older fetus agree with those well-known histochemical properties of the adult pancreas.

Except McAlpine[11)] and Hultquist and Thorell[12)], the fetal pancreas was not so often treated as the subject of histochemical studies in the past, and rather cursory accounts were made in the general observations on the distribution of phosphatases in the fetal organs by Moog[29)], Rossi *et al.*[27)28)] and others. Our study presented more details in the chronological alterations in some histochemical reactions of the developing pancreas, and the distribution of alkaline phosphatase observed was in an excellent agreement with McAlpine's findings in fetal and neonatal rats.

The most important finding in our study was the markedly enhanced activity of alkaline phosphatase in the primitive cells of both the primary and the secondary islets, and by this characteristic localization of the enzyme, as in the case of rat described by McAlpine, we could detect an initial appearance of the primitive islet cells about half a day before they became apparent in the sections treated with routine histological staining. In our preparations, however, such an intense activity of the enzyme is fairly restricted to the primitive cells which are just in the way of initial differentiation, and after that, they usually tend to lose their specific enzymic activity. Only the primary islets retain their slight reactivity until 13½ days, but with the formation of the band-like large mass of the islet, most of the islet cells lose their selective stainability. In the specimens ranging from the 15th to 16th day, the islet cells resumed temporarily a faint activity of alkaline phosphatase coinciding with the appearance of the beta granules in the greater number of the cells.

The slightly phosphatase-positive cells in the peripheral area of the fully developed islet may be the primitive alpha cells as considered so by McAlpine. It is very possible to think that the beta cells precede the alpha cells in the

development, since the most of the islet cells have been found containing beta granules on the 18th day, while the phosphatase-active peripheral cells have been observed in the specimens of later stage. Such a preceding formation of the beta cell also coincides with the findings of McAlpine, Hard[30], Bencosme[8], Munger[31] and others. So, as has been described by Bencosme, the primitive cells of the secondary islet found arranged in the walls of ducts in our preparations may therefore most likely correspond to the primitive beta cells.

The acinar cells also revealed distinct activity of alkaline phosphatase at the initial stage of the development showing an epithelial alignment and a convex bulging. It may be postulated, therefore, that the activity of this enzyme participates to some extent in the metabolism of cells underlying the morphological specialization during the differentation and maturation. Such a probable relationship between morpho- and histogenetic process and the activity of the enzyme was first described by Moog[27] in chick embryos, and later, extensively discussed by Rossi *et al.*[32], Mulnard[33] and Borghese[34]. McAlpine also suggested that this enzyme is more closely linked to the differentation of the islet cell than to the proliferation of the cell.

The most initial stage of the differentiation of the primary islet cells from the primitive cell cord of the pancreatic anlage was also characterized by an intense cytoplasmic basophilia besides the distinct positiveness of alkaline phosphatase described above. A half day later, however, this basophilia rapidly diminished and its intensity became feebler than that of the other cells of the pancreatic anlage. After that, all the cells of the primary and secondary islets retained a low content of RNA throughout the whole stage of their pre-natal development, as has been already described by Hultquist and Thorell[12], and by Hinrichsen (unpublished data cited in Vendrely's review[21]). But, unlike the findings of Hultquist and Thorell, there was little evidence that the primitive islet cells found arranged in tubular epithelium carried a high content of RNA, and our results rather suggested that these primitive islet cells in later stage were less basophilic than the proper duct cells. Recently, Munger[31] described in his electronmicroscopical study on the developing pancreas of mouse that the primitive islet cells of 13-day-embryos appeared "comparatively dense due to numerous ergastoplasmic granules filling the cytoplasm", while in those from 15-day-specimens the ergastoplasmic granules decreased in amount. Then, it may be deemed that the staining technic for the cytoplasmic RNA with pyronin and gallocyanin used in this study failed to demonstrate such a slight accumulation of RNA in the primitive cells of the secondary islets.

The acinar cells on the other hand revealed a large content of RNA from the first stage of their development. It was noticed that every small acinus in the very early stage of development, even those consisting of a small number of cells and just protruding out from the duct, showed a high content of RNA in their

cytoplasm and nucleoli. But an intense basophilia which lasts into the later stage of development is not merely related to the growth and differentiation but also to the formation of the zymogen granules. This assumption may also be supported by an apparent reduction of the cytoplasmic basophilia accompanying the rapid appearance of the zymogen granules during 16 to 17½ days, such an inverse trend in the amount of cytoplasmic RNA and the zymogen granules being in fairly good agreement with the densitometric observations of Oram[35] on the secretory cycle of the pancreatic acinar cells.

From these findings, it may be concluded that RNA also participates in the cellular differentiation during the ontogenesis, as has been pointed out by Caspersson and Thorell[36]. On this problem, basing mainly on the histo- and cytochemical data, Brachet[19][20][37][38][39] and Caspersson[21][22] discussed repeatedly, and they mostly emphasized a possible participation of RNA in the intracellular synthesis of protein. Recent progress in the bicohemical researches on the biological role of nucleic acids also tends to substantiate this hypothesis, and extensive reviews on this subject have recently been made by Brachet[38][39]. In this study, however, the activity of alkaline phosphatase is apparently more closely linked to the differentiation, especially in the endocrine cells of the pancreas. In spite of occasional irregular results, the activity of acid phosphatase also seemed to be related to some extent to the initial differentiation and further maturation of the islet cells. Since the iselts of adult animal also show an evident activity of this enzyme (Noback and Montagna[26]), it may take part in the specific function of the islet cell, too. The activity of lipase is unquestionably related to the zymogen granules. It has been demonstrated only in the excretory cells of the older specimens having the granules.

Although the chronological alterations in histochemical properties during the development of the endocrine and the exocrine cells of the pancreas was the primary concern in this report, other problem dealing with the histogenesis, especially that of the islets of Langerhans was also studied. In general, the histogenetical process observed in this study was very similar to the description of Yasunaka[1] on mouse, and the two stages of the islet formation were confirmed. In this study, the characteristic localization of alkaline phosphatase made it very obvious that the cells of the primary islets were derived from the primitive cord of the dorsal pancreas while those of the secondary islets principally originated from the walls of the pancreatic tubules. Any intimate relation between the developing primary islets and the sympatheitc ganglia described by Van Campenhout[10] has not been confirmed. There was little evidence about the acinar origin of the islet cells except only the occasional and questionable existence of phosphatase-positive cells in the primitive acini. As has been mentioned above, the primitive islet cells found arranged in the duct epithelia have been regarded as the primitive beta cells for the time being, but further studies should be done

on this problem, since some of these cells have been looked upon as the primitive alpha cells by some authors such as Ferner and Stoeckenius[7], and Hultquist and Thorell[12].

SUMMARY

The intracellular distribution of alkaline and acid phosphatases, lipase and RNA in the developing pancreas of fetal mouse was observed histochemically. During the course of this study, general observations on the histogenesis of the pancreas, especially that of the islets of Langerhans, were also carried out. The findings may be summarized as follows:

1. Two successive developmental stages of the islets, the primary and the secondary, were confirmed. No degeneration of the primary islets was however established.

2. The primary islets initially appeared in the dorsal anlage of the pancreas of 10 days as the masses of cells showing an intense basophilia and a high phosphatase activity. The amount of cytoplasmic RNA was rapidly decreased thereafter.

3. The secondary islets appeared in the dorsal anlage of 12½ days, while those in the ventral anlage was first observed one or half a day later. The tubular origin of the secondary islets was confirmed, and the primitive cells of this generation of the islets were characterized by an intense activity of alkaline phosphatase. No specific accumulation of RNA in these cells was detected by the staining with pyronin and chrome alum gallocyanin.

4. The activity of acid phosphatase was also fairly restricted to the islets and the duct from the early stage of development, and the activity lasted until the latest date of the intra-uterine life.

5. The activity of lipase was observed only in the fully developed excretory cells having the zymogen granules.

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Explanation of Figures

Plate I

Fig. 1. The dorsal pancreatic anlage of a 10-day-embryo. The anlage mainly consisted of a thick epithelium forming an evagination of the mid-gut. From the distal part (upper in the figure) of the anlage lumps of intensely basophilic cells are migrating out. Stained with methyl green and pyronin.

Fig. 2. Low-power view of the dorsal anlage of 10½ days. The anlage still retains a small cavity. At the distal end of it, small primary islets are attached, of which cells are characterized by a diffuse and faint cytoplasmic basophilia, palely stained vesicular nucleus, and indistinct boundary of the cell body. Methyl green and pyronin.

Fig. 3. The dorsal anlage of 12 days, showing an extensive progress of ramification of the tubules and formation of the primitive acini. From the walls of the tubules faintly basophilic cells of the secondary islets are being formed (SI). Some cut surfaces of the primary islet (PI) are also seen between, and partially connecting with, the tubules and acini. Hematoxylin and eosin.

Fig. 4. A low-power view of the dorsal pancreas of 14½ days stained with methyl green and pyronin. All the acini including those consisting of a few primitive acinar cells reveal a very intense affinity to pyronin. The epithelial cells of the duct rank the next in the cytoplasmic basophilia, while the islet shows generally only a limited affnity to pyronin except more perceptible staining in some of the small secondary islets attached to the duct.

Plate II

Fig. 5. Distribution of alkaline phosphatase in the distal part of the dorsal pancreatic anlage of 10 days. Most of the parenchymal cells are stained only faintly, among which intensely stained primitive cells of the primary islet are found intermingled. The mesenchymal tissue surrounding the pancreatic anlage exhibits a moderate activity of the enzyme.

Fig. 6. The dorsal pancreas of the 12-day-specimen stained for alkaline phosphatase. The superfiical cells in the form of a convex columnar epithelium show an enhanced activity of the enzyme. The cell groups appointed by arrows form a primary islet, which is a large mass of the cells communicating with each other in the other sections. The more proximal part of the anlage surrounding a diverticle reveals weaker reaction, in which small number of highly stained cells are found scattered.

Fig. 7. The dorsal pancreas of 13 days and 6 hours. The primitive islet cells scattering in the walls of the tubules and some of the developing acini show a distinct activity of alkaline phsophatase.

Plate III

Fig. 8. Low-power view of the dorsal pancreas of 14 days stained for alkaline phosphatase. Many primitive islet cells arranged in the duct walls are standing out by their distinct activity of the enzyme against the feebly stained ducts and acini.

Fig. 9. Higher magnification of the pancreatic duct of 15 days stained for the same enzyme. The intensely reactive primitive islet cells are found sigularly intercalated in the duct wall or projecting out from the duct forming small masses.

Fig. 10. Shows highly reactive centro-acinar cells found in the same specimen illustrated in the preceding figure.

Plate IV

Fig. 11. Acid phosphatase reaction in the dorsal pancreas of 12 days. The central part of the anlage including the duct and the developing islets is stained most intensely.

Fig. 12. The same enzyme in the pancreas of 16 days. The walls of the duct and the islets are most reactive, and the acini show a moderately intense reaction, too.

Fig. 13. The distribution of the same enzyme in the pancreas of 18½ days. The definite islet in the center of the figure and the ducts exhibit very strong enzymic activity, while the reaction of the excretory cells is conspicuously reduced.

Plate I

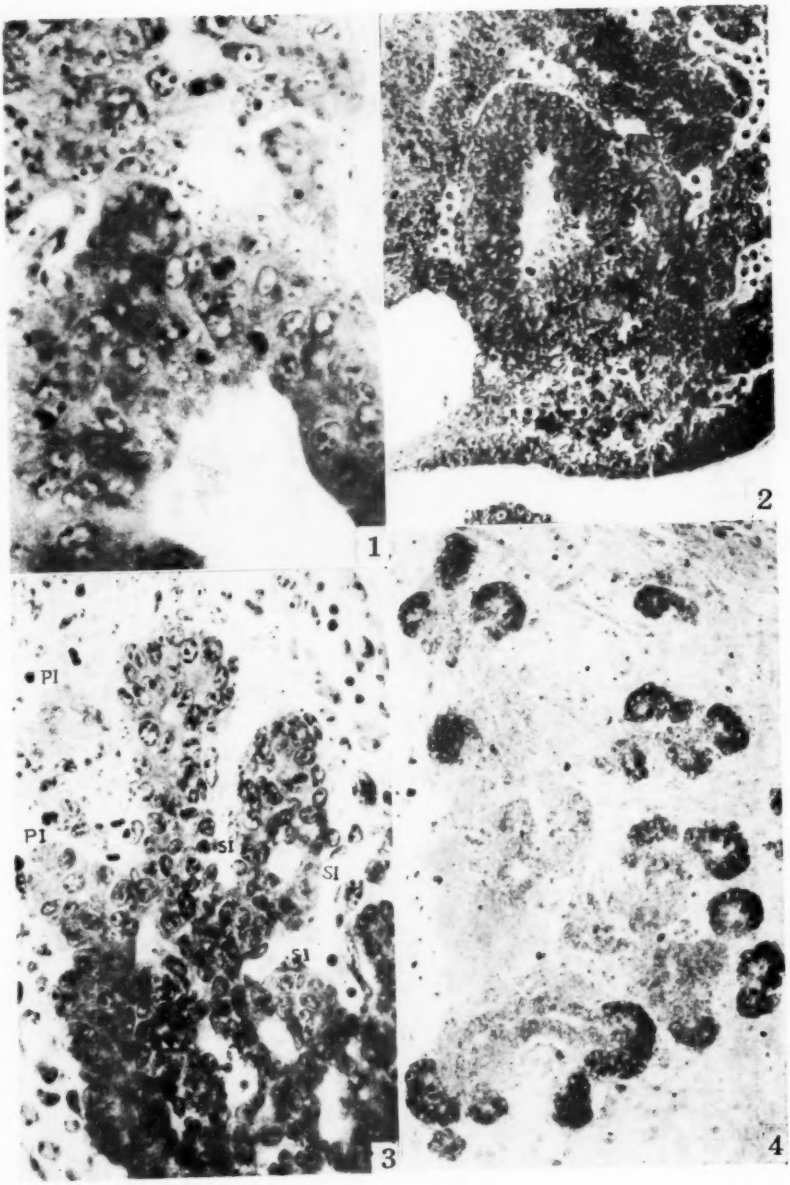


Plate II

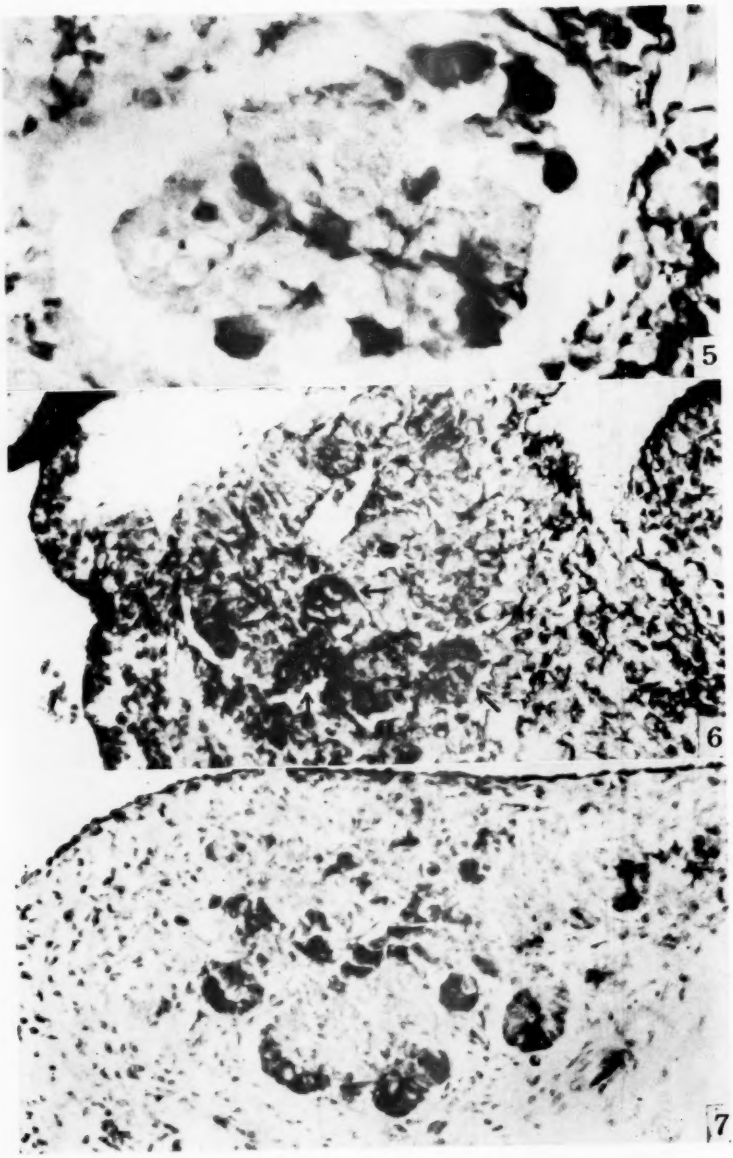


Plate III

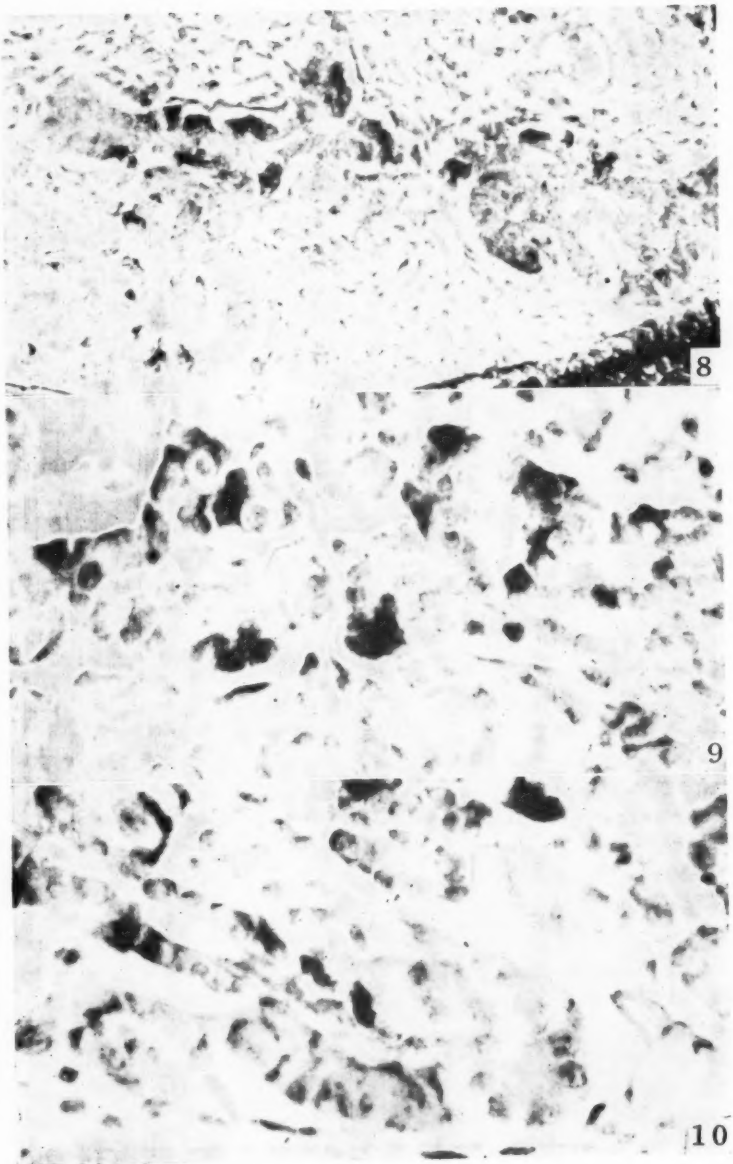
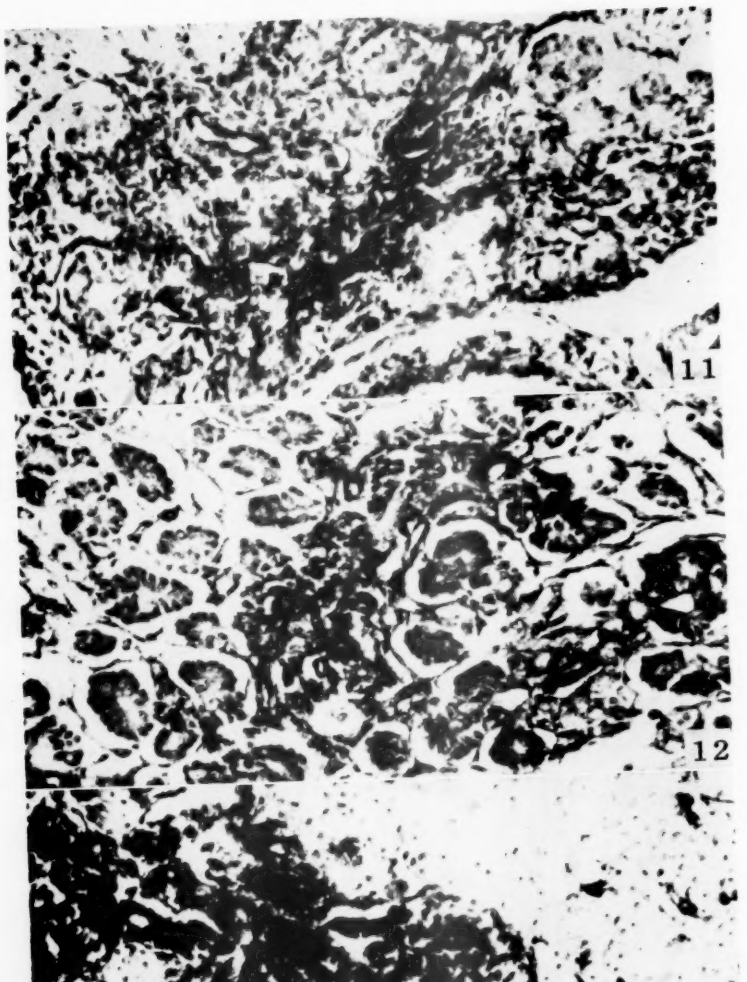


Plate IV



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Statistical Study on Relation between Breast Cancer and Lactation Period

First Report

A Comparative Study through Cumulative Frequency Distribution

By

Mitsuo Kamoi

From the Department of Public Health, Tohoku University, Sendai; Director: Prof. M. Segi

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The rate of deaths from breast cancer is generally observed considerably high in Western countries as compared with that in Japan. We have many reports of inquiries and animal experiments processed on the supposed relation of breast cancer and lactation conditions such as by Dr. Kogure,[1] Dr. Kuru,[2] Dr. Kawasaki,[3] Dr. Fujimori,[4] Dr. Ogata,[5][6] Dr. Segi,[7][8] Dr. Stocks[9][10] and others, but there are few records of statistical researches made in detial on the relation of the human lactation conditions to the growth of breast cancer.

I. MATERIALS AND METHODS

The investigation group of the Committee for Epidemiological Study on Cancer (organized in 1953 and at work through 1955 sponsored by the Ministry of Welfare and Public Health. Chairman: Dr. Tomosaburo Ogata) requested 33 university hospitals throughout Japan and the Hospital of Foundation for Cancer Research to answer the questionaires on their cancer patients. This kind of request was conducted twice and the filled questionaires were consolidated at the Department of Public Health of Tokhou University in charge of the tabulation and the analysis of the data. Beside this program, our Department of Public Health requested different hospitials in the Tohoku District and many practising midwives throughout the country to fill our own questionaires prepared in the same regard. From the recorded cases of breast cancer as answered in these two lines of questionaires, I have picked up for the materials of my specific study a total of 811 cases of women with the experience of live birth. (hereunder referred to as "vivipara")

For the controls, I have taken 1,239 cases of vivipara from the questionaires

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filled by officials of health centers (physicians or public health nurses) who conducted the work when the investigation group of the Comittee for Epidemiological Study on Cancer asked various health centers all over Japan to pick up cases from general women free from breast cancer and make direct inquiries to meet the prepared purpose.

The materials for my study have been limited to cover only vivipara, and in treating the mode of feeding, artificial feeding was excluded but mixed feeding has been considered as natural feeding.

No discrimination has been drawn in the type of delivery, single or plural. For example, in the case of twins, the mothers's lactation period has been set at the longer term when there was any difference in the duration of feeding the two.

The lactation period or term has been taken with month as the unit, counting days less than one month and 15 days and over as one month and regarding 14 days and less as zero. Accordingly, the lactation period zero (0) here includes the cases in which natural feeding did not continue over 14 days due to the death of babies or the change over to artificial feeding.

II. STUDY THROUGH THE TOTAL OF LACTATION PERIODS

With 811 breast cancer patients and 1,239 control cases classified by urban and rural districts and agegroup ; I have totaled the lactation periods a mother needed in all here deliveries and for my purpose set this total value as her lactation period. (hereunder referred to as "total lactation period(s)" or "t.l.p.")

TABLE I. Mean and Standard Deviation of Total Lactation Period

		Urban			Rural		
		Sample size	Mean and significance (month)	s.d. (month)	Sample size	Mean and significance (month)	s.d. (month)
30–39 years	Patient	97	44.6	28.6	111	57.1	35.7
	Control	52	40.3	25.8	75	56.4	12.6
40–49	Patient	190	54.1 **	38.4	167	76.7 **	49.2
	Control	165	64.8	18.2	222	87.5	17.6
50–64	Patient	142	61.7 **	44.0	104	76.7 **	48.4
	Control	309	81.5	16.0	416	100.8	60.0

** Denotes significance at 1% level.

As shown in Table I, both in urban and rural districts, a significant difference is seen between the mean values of t.l.p. (or t.l.p. means) of the patients and those of the controls in the groups for 40 and above years of age, but their difference is not significant in the 30–39 age-groups.

For further study, I have worked out a cumulative distribution regarding the total lactation periods, as is shown in Table II. It's graph is shown in Fig. 1.

TABLE II. Cumulative Frequency Distribution according to Total Lactation Period (Total: 100%)

Period (less than .... years)	Urban				Rural			
	40–49 years		50–64 years		40–49 years		50–64 years	
	P	C	P	C	P	C	P	C
1	8.9	9.7	9.9	2.6	6.6**	2.7	5.8	1.9
2	23.7	17.0	20.4	12.0	13.8*	9.5	14.6	8.4
3	31.1	27.9	30.3	20.1	24.6	18.9	17.5	12.0
4	47.9	40.0	40.8	28.5	34.7*	25.7	22.3	18.0
5	57.9	51.5	51.4**	39.2	47.3*	32.9	33.0	26.2
6	67.9	63.0	59.2*	45.0	52.1**	40.6	43.7	33.4
7	77.7	72.1	69.7*	53.4	61.1**	50.0	57.3**	41.8
8	85.3*	76.4	81.0*	61.8	68.9**	57.7	65.0**	50.0
9	90.0**	80.0	83.1	68.4	73.3**	67.6	77.7**	57.0
10	92.1*	83.6	87.3	75.4	80.8**	72.5	81.6**	66.1
11	94.7**	84.8	90.8**	83.2	88.6*	77.0	84.5**	74.0
12	97.4**	86.7	95.1*	87.4	91.0*	82.9	86.4	79.1
13	98.9**	89.7	97.9	91.9	93.4	88.3	90.4	82.7
14	99.5**	92.7	99.3	92.6	95.8	91.0	92.2	85.3
15	100.0	95.8	99.3	94.8	97.7	93.7	95.2*	87.7

P Denotes Patient. C Denotes Control.
* Denotes significance at the 5% level.
** Denotes significance at the 1% level.

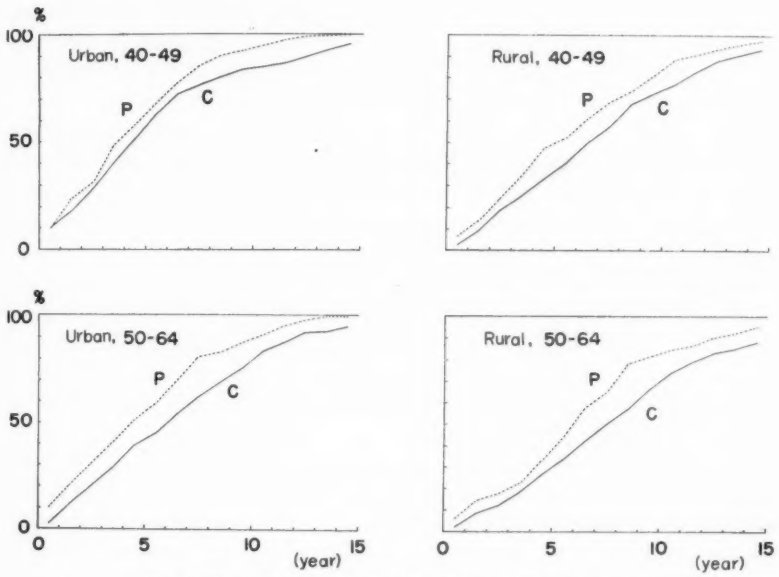

Fig. 1. Relative cumulative distribution according to the t.l.p. (total is 100%)

In the urban 40–49 age-group, for t.l.p. less than one year, 8.9 % is covered by the patients against 9.7 % by the controls, but the difference is not significant. For t.l.p. less than two years, the percentage covered by the patients is 23.7 % and that by the controls 17.0 %, the former standing larger by 6.7 %, but the difference is not significant. Such state that in percentage the patients stand larger than the controls with no significant difference remains up to the t.l.p. less than 7 years. From this we can conclude: "In the percentage of vivipara with the total lactation periods short, no significant difference is seen between the patients and the controls." Our terming "short" here stands on the following basis: In reference to Table I, for the urban 40–49 age-group, by media of 64.8 (month) as t.l.p. mean and 18.2 (month) as s.d. for the controls, we can get; "(Mean) – (s.d.) = 64.8 – 18.2 = 46.6." Now 46.6 months represent 3.6 years, and the total lactation period of 3.9 years are regarded "short" for this group.

Similarly we can get, 64.8 + 18.2 = 83.0 (month) = 6.9 (year). Then, the total lactation period of 6.9 years can be termed "long" for this group.

In the case of t.l.p. less than 8 years, the patients cover 85.3 % and the controls 76.4 % and the difference appears significant. When the total lactation periods exceed 8 years, the percentage of the patients is: 100–8.5.3 = 14.7 (%), and that of the controls: 100–76.4 = 23.6 (%). The difference stands significant. This is important in meaning. Now, 8 years differ from 6.9 years as termed "long" above, but we may safely conclude: "In the percentage of those with the total lactation periods long, a significant difference is seen between the patients and the controls".

This conclusion can be expressed in other words: "Breast cancer grows at lower rate to the vivipara holding the total lactation periods long than to those holding them not long". This exclusively true to the urban 40–49 age group. I do not want to repeat such different expressions on the same contents, but the relation is quite important.

For the urban 50–64 age-group, through media of 81.5 (month) as t.l.p. mean and 16.0 (month) as s.d. as in Table I, we can get 81.5 ± 16.0 and set the t.l.p. for the group with 8.1 years as long and 5.5 years as short. From this and the figures in Table II, we can conclude: "In the percentage of vivipara with the lactation periods either short or long, a significant difference is seen between the patients and the controls".

For the rural 40–49 age-group, from the result of 87.5 ± 17.6 and the figures in Table II, we can conclude that, in the percentage of those with t.l.p. either long or short, a significant difference is seen between the patients and the controls.

For the rural 50–64 age-group, no significant difference is found between the patients and the controls, where the t.l.p. is either long or short in our definition.

III. STUDY FROM THE LONGEST PERIOD OF LACTATION

With the materials and their classification just in the same line with the preceding part II, I have conducted a comparative study from the point of the longest period of lactation that a mother needed in feeding her babies. (hereunder referred to as the longest lactation period.)

The comparison of the mean values of the longest lactation periods made in varied groups corresponding to Table I has shown us that a significant difference between the patients and the controls exists only in the urban 50–64 age-group. Also the study made through the cumulative distribution has proved that a significant difference between the patients and the controls exists only in the urban 50–64 age-group and only when the longest lactation periods stand less than one year and when less than 4 years.

IV. STUDY THROUGH THE NUMBER OF DELIVERIES AND THE TOTAL LACTATION PERIODS

The number of deliveries or births and the total lactation periosd generally move in proportion. Table III shows the cumulative distribution regarding the number of live births. Appeared here common to the 4 groups is the fact that in smaller number of live births, the patients stand large in percentage.

TABLE III. Cumulative Frequency Distribution according to Number of Live Birth. (Total: 100%)

Number of live birth (less than)	Urban				Rural			
	40–49 years		50–64 years		40–49 years		50–64 years	
	P	C	P	C	P	C	P	C
1	21.6**	10.3	16.2	11.3	16.1*	9.5	15.4*	7.5
2	35.3	20.6	33.1**	20.7	31.1*	21.2	22.1	17.1
3	50.5	44.2	52.1**	36.2	45.5**	31.5	32.7	28.6
4	70.5	66.1	64.1**	48.9	56.9*	44.6	49.0	40.4
5	84.7	78.8	77.5**	63.1	69.5	61.7	61.5	55.3
6	92.1*	84.8	90.1**	74.1	83.8	75.7	76.0	69.0
7	97.4*	92.7	94.4**	83.8	92.2	86.5	86.5	78.4

P Denotes Patient. C Denotes Control.
* Denotes significance at the 5% level.
** Denotes significance at the 1% level.

Different combinations of the number of live births with the total lactation periods have revealed noticeable facts as shown in Table IV. As the grouping is further detailed the number of object cases is reduced, but we can notice a trend that a significant difference between the patients and the controls is found in such groups where the total lactation periods are short and the number of live

TABLE IV. Cumulative Frequency Distribution according to Number of Live Birth, Classified by Total Lactation Period. (Total: 100%)

Number of live birth (less than ....)	Total lactation period (years)							
	0–3		3–6		6–9		9–	
	P	C	P	C	P	C	P	C
	the urban 40–49 age-group							
(Sample size)	(59)	(46)	(70)	(58)	(42)	(28)	(19)	(33)
1	61.0**	34.8	5.7	1.7	2.3	0.0	0.0	0.0
2	88.1**	60.9	14.3	6.9	11.9	7.1	0.0	0.0
3	93.2	80.4	44.3	41.4	16.7	35.7	15.8	6.1
4	94.9	93.5	80.0	79.3	42.9	57.1	21.1	12.1
5	100.0	93.5	95.7	93.1	61.9	82.1	47.4	30.3
6		95.7	98.6	94.8	78.6	92.9	73.7	45.5
7		100.0	100.0	98.3	97.6	100.0	78.9	66.7
8				100.0	100.0		89.5	81.8
	the urban 50–64 age-group							
(Sample size)	(43)	(62)	(41)	(77)	(34)	(73)	(24)	(97)
1	46.5	45.2	4.9	9.1	2.9	0.0	0.0	0.0
2	83.7	69.4	24.4	20.8	2.9	5.5	0.0	1.0
3	100.0*	83.9	58.5	50.6	14.7	16.4	8.3	9.3
4		90.3	82.9	75.3	29.4	32.9	16.7	13.4
5		90.3	92.7	92.2	47.1	54.8	54.2*	28.9
6		93.5	95.1	97.4	82.3	72.6	75.0**	44.3
7		95.2	95.1	97.4	97.1	87.7	79.2	62.9
8		96.8	97.6	98.7	97.1	94.5	83.3	79.4
	the rural 40–49 age-group							
(Sample size)	(41)	(42)	(46)	(48)	(36)	(61)	(44)	(71)
1	56.1	42.9	8.7	6.2	0.0	0.0	0.0	0.0
2	82.9	76.2	37.0	22.9	0.0	4.9	2.3	1.4
3	90.2	88.1	71.7*	47.9	2.8	11.5	11.4	4.2
4	92.7	90.5	87.0	72.9	25.0	29.5	18.2	11.3
5	92.7	92.9	93.5	89.6	58.3	55.7	31.8	29.6
6	95.1	100.0	100.0	91.7	80.6	77.0	59.1	49.3
7	97.6			97.9	94.4	91.8	77.3	66.2
8	97.6			100.0	94.4	100.0	86.4	77.5
	the rural 50–64 age-group							
(Sample size)	(18)	(50)	(27)	(89)	(36)	(98)	(23)	(179)
1	55.6	46.0	18.5	7.9	2.8	1.0	0.0	0.0
2	72.2	80.0	29.6	27.0	5.6	7.1	0.0	0.0
3	83.3	92.0	48.1	48.3	13.9	19.4	4.3	6.1
4	94.4	96.0	70.4	69.7	33.3	40.8	13.0	10.1
5	100.0	100.0	81.5	89.4	50.0	58.2	26.1	24.0
6			96.3	95.4	75.0	84.7	34.8	39.1
7			100.0	98.9	88.9	91.8	56.5	56.4
8					94.4	99.0	65.2	73.7

P Denotes Patients. C Denotes Controls.
* Denotes significance at the 5% level.
** Denotes significance at the 1% level.

births is small.

Considering the fact that in Japan the number of deliveries by a mother is generally larger and the lactation period longer than the case in Western countries, we may count the trend shown in our study as a reason that the growth of

breast cancer is smaller in number in Japan than in Western countries.

V. SUMMARY

With vivipara 811 breast cancer patients and 1,239 control cases as the materials, I have studied the relation of the lactation periods to the growth of breast cancer, and now think to have reached the following conclusion: (The matter of the longest lactation period is not regarded instrumental to the growth of breast cancer).

In the 30–39 age-group, the total lactation periods little affect the growth of breast cancer, but their relation appears conspicuous in the groups over 40 and above years of age. As a general trend, it is observed that a close relation exists between the shorter total lactation period and the increased growth of breast cancer on one hand and the longer total lactation period and the decreased growth of breast cancer on the other. But this relation differently appears in degree in accordance with the variety of groups on age and districts, urban and rural.

The number of live births and the total lactation periods hold an inseparable relation, and I have got a valuable suggestion that the smaller number of live births with shorter total lactation periods may be instrumental to the growth of breast cancer.

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Statistical Study on Relation between Breast Cancer and Lactation Period

Second Report
Adoption of Square Root Measure

By

Mitsuo Kamoi

From the Department of Public Health, Tohoku, University Sendai; Director: Prof. M. Segi

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Following the practice in my preceding report, I have taken up only the women with the experience of live birth (vivipara) as the materials for the current study. Standing on the fact found in the preceding study that the total lactation period or the total of a mother's feeding period she needed in each delibery has a close relation to the growth of breast cancer, I have taken in the currents tudy this total lactation period (t.l.p.) as measure for lactation period.

In this study, the number of breast cancer cased has been increased to 879, of which 147 cases with the previous history of mastitis have been checked up for the object of my specific study.

ADOPTION OF SQUARE ROOT MEASURE

When the coordinate axis of the total lactation periods is divided into equal intervals by the ordinary measure of months grouping, we can get a frequency distribution of vivipara as in Fig. 1, (1), which shows a considerable departure from normality. Is'nt it possible to get a normal distribution of vivipara through a change of the measure of total lactation period? In the first place, we can take logalithm of t.l.p. in application of the method known in economic statistics as the Gibrat ,principle. After varied trials, I have found that the measure best meeting the purpose lies in the use of the square root of t.l.p.. Fig. I, (2) shows the frequency distribution of vivipara made from the same materials for (1), with the abcissae of t.l.p. divided into equal intervals in our square root measure.

I have made examinations to find if our data prepared through the s.r.m. (square root measure) can represent samples from the normal population. Among various ways of checking, I have taken the following method: In

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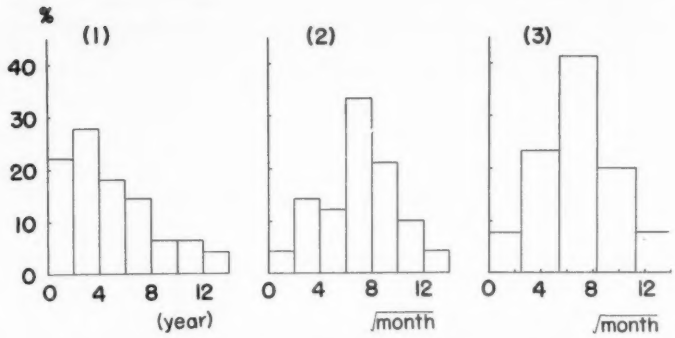


Fig. 1. Relative frequency distributions of patients in the urban 45–49 age-group, according to the total lactation periods in ordinary and square root measure.

normal population, the relative frequency appears as 6.7 %, 24.2 % 38.3 %, 24.2 % and 6.7 % for the 5 unequal intervals taken by 4 values of (mean)$\pm\frac{1}{2}$ (s.d.) and (mean)$\pm\frac{3}{2}$(s.d.). This forms the basis for getting a theoretical frequency distribution. By media of the mean value (mean) and the standard deviation (s.d.) as taken from our s.r.m. for total lactation period, we can get 4 different values and by them 5 intervals, and thus form our frequency distribution for our materials. This represents an observed farequency distribution. Fig. 1, (3) indicates the frequency distribution made in this method from the materials same for the foregoing two figures. I have made χ^2-test to see if our observed frequency distribution can stand with goodness of fit with the theoretical frequency distribution.

The results of our χ^2-test are summarized as follows: We must test normality as regards 42 groups in Table I, but we excluded from our test 9 groups of sample size 12 and less. Our test was also made to some groups with sample size 13 and over, each distributed into 3 intervals provided by media of the 2 values of (mean)$\pm\frac{1}{2}$(s.d.). The result has shown us that there is only one group or the urban 60-64 age-group where the calculated value of chi-square exceeds 5% value of χ^2-distribution. Even of this group, when the first interval holding the observed frequency 2 is put together with the next interval, the value of chi-square falls short of 5% value of the χ^2-distribution with the degree of freedom at 3. We have also found that there are 5 groups where the calculated chi-square value in each case falls short of 95% value of χ^2-distribution.

From these results, we have come to take s.r.m. as the measure of total lactation period. Let us see the effect of our measuring the total lactation periods by s.r.m., for instance, regarding the extension of the period one year. For the

TABLE I. (No. 1) Frequency Distribution of Breast Cancer Patients without Mastitis History and Controls according to Total Lactation Period in Square Root Measure

T.l.p. in s.r.m. ($\sqrt{\text{month}}$)	30–34 years		35–39 years		40–44 years		45–49 years		50–54 years		55–59 years		60–64 years	
	P	C	P	C	P	C	P	C	P	C	P	C	P	C
Urban														
Total	40	21	51	31	70	82	90	83	58	119	36	101	27	89
0– 1	1	—	2	1	3	8	2	2	2	1	2	1	—	—
1– 2	—	1	1	1	—	1	2	—	1	1	2	1	2	—
2– 3	—	1	—	—	3	1	3	2	2	1	2	1	—	1
3– 4	4	2	5	3	6	5	10	2	4	8	2	6	2	1
4– 5	8	5	5	5	9	7	7	8	6	6	3	7	5	10
5– 6	8	2	6	2	2	2	4	8	3	7	1	3	2	7
6– 7	9	7	13	6	14	19	21	7	11	15	5	9	2	12
7– 8	5	2	9	4	6	8	9	17	9	14	3	6	1	11
8– 9	2	1	2	5	14	10	8	10	5	17	6	13	4	4
9–10	3	—	2	3	5	6	11	9	4	12	3	19	6	10
10–11	—	—	4	1	6	4	6	3	6	16	3	16	—	9
11–12	—	—	1	—	2	2	3	2	5	10	2	6	1	9
12–13	—	—	1	—	—	6	4	7	—	5	1	8	2	7
13–14	—	—	—	—	—	3	—	1	—	4	—	3	—	4
14–15	—	—	—	—	—	—	—	2	—	2	—	2	—	3
15–16	—	—	—	—	—	—	—	3	—	—	1	—	—	1
16–17	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17–18	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rural														
Total	33	28	72	47	81	114	75	108	45	166	32	112	21	138
0– 1	1	—	3	2	4	2	4	2	1	3	1	—	1	2
1– 2	—	—	—	1	1	—	1	—	1	—	—	2	1	—
2– 3	—	—	—	—	—	1	1	—	2	1	—	—	—	—
3– 4	1	5	4	1	3	6	7	3	—	6	2	3	1	6
4– 5	8	5	4	7	7	17	7	4	5	6	2	8	1	1
5– 6	4	2	7	2	5	3	2	4	—	4	3	4	—	4
6– 7	7	7	20	7	16	13	9	12	5	21	2	7	4	8
7– 8	5	5	5	4	6	7	8	8	3	14	3	8	3	12
8– 9	3	2	11	9	9	17	8	11	7	24	9	10	2	16
9–10	2	1	8	3	6	16	7	14	9	18	5	15	3	20
10–11	2	1	6	6	7	11	14	14	4	27	3	20	1	17
11–12	—	—	2	1	7	6	2	13	1	16	—	7	2	19
12–13	—	—	2	3	6	8	5	15	3	10	1	10	2	14
13–14	—	—	—	1	2	4	—	3	1	4	1	9	—	6
14–15	—	—	—	—	1	1	—	3	3	6	—	8	—	5
15–16	—	—	—	—	—	—	—	2	—	4	—	—	—	6
16–17	—	—	—	—	1	2	—	—	—	1	—	—	—	2
17–18	—	—	—	—	—	—	—	—	—	1	—	1	—	—

vivipara with t.l.p. one year, the increase by s.r.m. is $\sqrt{2} - \sqrt{1} = 0.41$, whereas for the vivipara with the t.l.p. 10 years, the increase by s.r.m. comes as $\sqrt{11} - \sqrt{10} = 0.15$ or less than a half of the former.

COMPARISON OF MEANS OF TOTAL LACTATION PERIODS TAKEN BY S.R.M.

The mean and s.d. taken by s.r.m. for each group are as shown in Table II. These values have been calculated not from the figures in Table I but on another table of more detailed distribution made with month as the unit.

FABLE I. (No. 2) Frequency Distribution of Breast Cancer Patients with Mastitis History according to Total Lactation Period in Square Root Measure

T.l.p. in s.r.m. (month)	Urban							Rural						
	30–34	35–39	40–44	45–49	50–54	55–59	60–64	30–34	35–39	40–44	45–49	50–54	55–59	60–64
Total	7	13	18	21	11	8	6	6	13	12	15	12	4	1
0– 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1– 2	—	—	1	—	—	—	—	—	—	—	—	—	—	—
2– 3	—	1	—	—	—	—	—	—	—	—	—	—	—	—
3– 4	1	1	4	2	—	—	—	—	—	—	—	—	—	—
4– 5	1	2	1	2	—	1	—	—	—	—	—	—	—	—
5– 6	2	—	1	—	1	1	—	1	1	2	—	—	—	—
6– 7	2	2	6	1	2	—	4	2	2	2	4	3	—	—
7– 8	—	4	—	7	—	2	—	1	3	1	2	1	—	—
8– 9	—	2	1	1	4	2	1	1	1	2	3	3	—	—
9–10	—	1	2	7	1	2	—	—	3	3	4	3	—	—
10–11	1	—	—	1	1	—	—	1	2	1	1	1	1	—
11–12	—	—	1	—	—	—	—	—	—	—	—	1	—	—
12–13	—	—	—	—	2	—	1	—	1	—	—	—	1	1
13–14	—	—	1	—	—	—	—	—	—	1	—	—	1	—
14–15	—	—	—	—	—	—	—	—	—	—	1	—	—	—
15–16	—	—	—	—	—	—	—	—	—	—	—	—	1	—

The s.d. shown in Table II represents the square root of the unbiased estimate of variance. For the significance test of difference of means, we used the t-distribution table through the Welch method.

Let us contrast the breast cancer patients with no mastitis history with the controls. In urban districts, in the groups under 45 years of age, no significant difference is shown between our s.r.m. means for the patients with no mastitis history and those for the controls, whereas in the groups over 45 years of age, the s.r.m. means for the patients stand small with a significant difference in contrast with those for the controls.

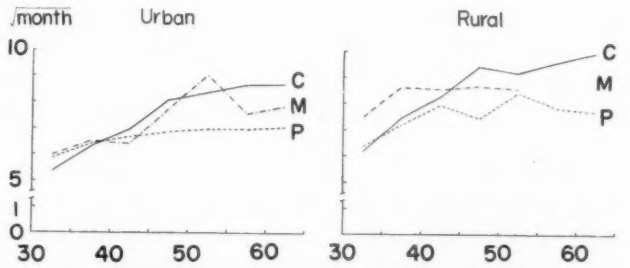


Fig. 2. The s.r.m. mean of total lactation periods.
P Denotes the paitents with no mastitis history.
M Denotes the patients with mastitis history.
C Denotes the controls.

TABLE II. Mean and Standard Deviation of Total Lactation Periods in Square Root Measure, Significance of Difference of Means

Age-group	Urban						Rural					
	Sample size	Mean in s.r.m.	S.D. in s.r.m.	Significance of Difference of Means			Sample size	Mean in s.r.m.	SD. in s.r.m.	Significance of Difference of Means		
				P&C	M&C	P&M				P&C	M&C	P&M
30–34				—	—	—				—	—	—
P	40	5.9	1.9				33	6.4	2.2			
M	7	6.0	2.1				6	7.5	1.9			
C	21	5.4	1.9				38	6.1	1.9			
35–39				—	—	—				—	—	—
P	51	6.4	2.6				72	7.2	2.6			
M	13	6.5	2.1				13	8.6	2.1			
C	31	6.3	2.4				47	7.5	3.1			
40–44				—	—	—				—	—	—
P	70	6.6	2.8				81	7.9	3.4			
M	18	6.4	3.1				12	8.5	2.3			
C	82	6.9	3.5				114	8.3	3.3			
45–49				*	—	—				**	—	—
P	90	6.9	2.9				75	7.4	3.3			
M	21	7.7	2.1				15	8.6	2.2			
C	83	8.1	3.3				108	9.4	3.0			
50–54				**	—	—				—	—	—
P	58	7.0	2.9				45	8.4	3.4			
M	11	9.0	2.4				12	8.5	1.7			
C	119	8.3	3.0				166	9.2	3.2			
55–59				*	—	—				**	...	...
P	36	7.0	3.7				32	7.8	2.8			
M	8	7.6	1.7				4	12.8	2.1			
C	101	8.6	3.0				112	9.6	3.3			
60–64				*	—	—				**	...	...
P	27	7.0	3.0				21	7.7	3.2			
M	6	7.8	2.3				1	(12.2)	—			
C	89	8.7	3.1				138	9.9	3.2			

* Denotes significance at the 5% level.
** Denotes significance at the 1% level.

In rural districts, in the groups under 45 years of age, as in the case of urban districts, no significant difference is shown between the s.r.m. means for the patients and those for the controls, but in the groups over 45 years of age, with the exception of the 50–54 age-group, the s.r.m. means for the patients stand small compared with those for the controls with a significant difference at the level of 1%.

As a general trend common to urban and rural districts, we may note that, in the groups over 45 years of age, the s.r.m. means for the patients with no mastitis history stand smaller than those for the controls with a significant difference, but in the groups under 45 years of age, no significant difference is shown between the s.r.m. means for the patients and those for the controls.

When the patients with mastitis history are compared with the patients with no mastitis history in regard to our s.r.m. means of t.l.p., we can see that in urban

districts, there is a trend in the groups over 45 years of age that the s.r.m. means for the patients with mastitis history stand large compared with those for the patients with no mastitis history, but the difference is not significant.

In rural districts, there appears a trend in all the groups over 30 years of age that the s.r.m. means for the patients with mastitis history stand larger than those for the patients with no mastitis history. But the fact that no significant difference is observed in the s.r.m. means for the two may be considered, just as in the case of urban districts, due to the small number of the taken cases of the patients with mastitis history.

Also the fact that no noticeable feature of difference is found between the s.r.m. means for the patients with mastitis history and those for the controls may be considered due to the small number of the taken cases of the patients.

SUMMARY

We have found that in setting the total lactation period of vivipara we can use its square root as its measure and produce normal distribution in our frequency distributions regarding breast cancer patients and controls.

Both in urban and rural districts, the s.r.m. means of total lactation periods for the breast cancer patients with no mastitis history and those for the controls show no noticeable difference in the groups under 45 years of age, but those for the patients stnad smaller than those for the controls in the groups over 45 years of age.

According to our data, of the total number of breast cancer patients, 16.7% is covered by those with mastitis history. There is an observed trend that the s.r.m. means of total lactation periods for the breast cancer patients with mastitis history stand large as compared with those for the patients with no mastitis history.

Tohoku J. Exper, Med., 1960, 72, 72-77

Statistical Study on Relation between Breast Cancer and Lactation Period

Third Report
Study through Relative Liability

By

Mitsuo Kamoi

From the Department of Public Health, Tohoku University, Sendai; Director: Prof. M. Segi

(Received for publication, December 14, 1959)

Through the same materials as in the second report, I will make a study on the quantitative relation of total lactation periods (t.l.p.) and breast cancer. The current study is greatly helped by the finding that our square root measure (s.r.m.) for total lactation periods can produce a normal frequency distribution for our objects.

As the breast cancer cases with mastitis history are small in number, I have shelved them from the current study, and the expression of "patients" in this report all means the breast cancer patients with no mastitis history.

METHODS

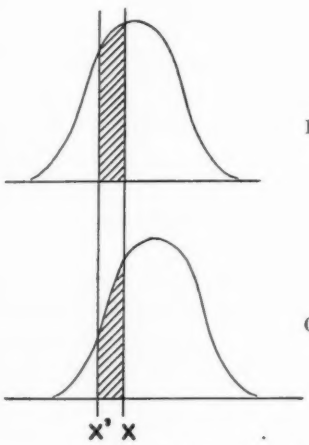
I wish to modify the method of relative liability, which was introduced by Woolf,[11] Haldane[12] and Edwards[13]. As shown in Table I, the value l_i indicates

TABLE I. Method of Relative Liability to Breast Cancer according to Total Lactation Period in Square Root Measure. (Urban, 35-39 age group)

Total lactation period in s.r.m.	Number of patients	Number of controls	Ratio	R.L.
Total	p	c	$p/c=r$	$r/r=1$
0-1	p_1	c_1	$p_1/c_1=r_1$	$r_1/r=l_1$
1-2	p_2	c_2	$p_2/c_2=r_2$	$r_2/r=l_2$
2-3	p_3	c_3	$p_3/c_3=r_3$	$r_3/r=l_3$
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
$(i-1)$ i	p_i	c_i	$p_i/c_i=r_i$	$r_i/r=l_i$
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

the relative liability (R.L.) and has the meaning: in the urban 35–39 age-group, the breast cancer grows l_i times larger (in the rate) by the vivipara whose t.l.p. lies in the s.r.m. interval $(i-1,\ i)$ compared with the whole vivipara (in the urban 35–39 age-group).

I showed in the second report that the square root measure (s.r.m.) for total lactation period (t.l.p.) can produce approximately a normal frequency distribution for our object. Now in the urban 35–39 age-group, let the population distribution function of breast cancer cases with no mastitis history and that of controls be as follows respectively:



Patients: $\dfrac{1}{\sqrt{2\pi}\,\sigma_1}\exp\left\{-\dfrac{(x-m_1)^2}{2\sigma_1^2}\right\}$

Controls: $\dfrac{1}{\sqrt{2\pi}\,\sigma_2}\exp\left\{-\dfrac{(x-m_2)^2}{2\sigma_2^2}\right\}$

Fig. 1. Normal frequency distribution.

$$\text{Patients}: \quad \frac{1}{\sqrt{2\pi}\,\sigma_1}\exp\left\{-\frac{(x-m_1)^2}{2\sigma_1^2}\right\} = \varphi_1(x)\,,$$

$$\text{Controls}: \quad \frac{1}{\sqrt{2\pi}\,\sigma_2}\exp\left\{-\frac{(x-m_2)^2}{2\sigma_2^2}\right\} = \varphi_2(x)\,,$$

then we have

$$\int_{-\infty}^{\infty}\varphi_i(x)\,dx = 1\,, \qquad (i=1,2)\,.$$

For the sake of explanation, let us consider that all figures in Table I are concerned with the populations, then we have

$$p\int_{-\infty}^{\infty}\varphi_1(x)\,dx = p\,, \qquad c\int_{-\infty}^{\infty}\varphi_2(x)\,dx = c\,,$$

$$p\int_{i-1}^{i}\varphi_1(x)\,dx = p_i\,, \qquad c\int_{i-1}^{i}\varphi_2(x)\,dx = c_i\,.$$

Hence we get

$$l_i = \frac{r_i}{r} = \frac{p_i/c_i}{p/c} = \frac{p_i}{c_i} \Big/ \frac{p}{c}$$

$$= \frac{p\int_{i-1}^{i} \varphi_1(x)\,dx}{c\int_{i-1}^{i} \varphi_2(x)\,dx} \Big/ \frac{p\int_{-\infty}^{\infty} \varphi_1(x)\,dx}{c\int_{-\infty}^{\infty} \varphi_2(x)\,dx} = \frac{\int_{i-1}^{i} \varphi_1(x)\,dx}{\int_{i-1}^{i} \varphi_2(x)\,dx}.$$

The ratio l_i is a relative liability corresponding to the internval $(i-l,\ i)$, but we may define the relative liability $l(x',\ x)$ corresponding to any interval $(x',\ x)$ by:

$$l(x',\ x) = \left(\int_{x'}^{x} \varphi_1(t)\,dt\right) \Big/ \left(\int_{x'}^{x} \varphi_2(t)\,dt\right).$$

And thus we may define the limiting function $l(x)$ by

$$\lim_{x' \to x} l(x',\ x) = \lim_{x' \to x} \left(\int_{x'}^{x} \varphi_1(t)\,dt\right) \Big/ \left(\int_{x'}^{x} \varphi_2(t)\,dt\right)$$

$$= \varphi_1(x)/\varphi_2(x)$$

$$= \frac{\sigma_2}{\sigma_1} \exp\left\{-\frac{(x-m_1)^2}{2\sigma_1^2} + \frac{(x-m_2)^2}{2\sigma_2^2}\right\} = l(x).$$

It will be evident that $l(x)$ has the similar meaning as that of l_i.

The values of limiting function $l(x)$ may be calculated for any value of x and describe a smooth curve, if we can know the values of 4 parameters m_1, m_2, σ_1 and σ_2. But we can get only the estimating values ("statistics") of these parameters. The statistics are thereby the s.r.m. means and s.d. concerning the patients and controls respectively. Using the statistics instead of 4 parameters, $l(x)$ has a property of "sampling deviation" in a sense. I shall have another chance to discuss this problems.

For the purpose of diminishing the sampling deviation of $l(x)$, it is desirable to enlarge the sample size. For example, the urban 35–39 age-group of patients (or controls) can be put together with the urban 40–44 age-group of patients (or controls) by reason of no significant difference between the s.r.m. mean for the former and that for the latter. It will be clear that this combined group, the urban 35–44 age-group in our data, is regarded as a sample from a normal population.

RESULTS

Within a same age-group of vivipara, the quantitiative relation of t.l.p. in s.r.m. and frequencies of breast cancer cases is analized by $l(x)$, as shown in Table II. Fig. 2 is described using Table II, the abcissae being scaled by the ordinary measure of t.l.p.. Attention is called to the relation R.L. = 1, whose meaning varies according to the classified age-groups in the urban and the rural districts.

TABLE II. Relative Liability ot Breast Cancer according to Total Lactation Period. (Evaluated by the Function $l(x)$)

Total lactation period		Urban			Rural		
in s.r.m.	in ordinary measure (month)	35–44 years	45–54 years	55–64 years	35–44 years	45–54 years	55–64 years
0	0	0.5	2.2	6.0	1.2	5.4	3.4
1	1	0.7	2.0	4.4	1.2	4.2	3.2
2	4	0.8	1.9	3.3	1.2	3.2	2.9
3	9	1.0	1.8	2.5	1.2	2.6	2.7
4	16	1.1	1.6	2.0	1.2	2.0	2.4
5	25	1.2	1.5	1.6	1.1	1.7	2.1
6	36	1.2	1.3	1.3	1.1	1.4	1.8
7	49	1.2	1.2	1.0	1.1	1.2	1.5
8	64	1.1	1.0	0.9	1.0	0.9	1.3
9	81	1.0	0.8	0.8	1.0	0.7	1.0
10	100	0.9	0.7	0.7	0.9	0.6	0.8
12	144	0.6	0.5	0.6	0.8	0.4	0.5
14	196	0.3	0.3	0.5	0.7	0.3	0.3
16	256	0.1	0.2	0.5	0.5	0.2	0.2

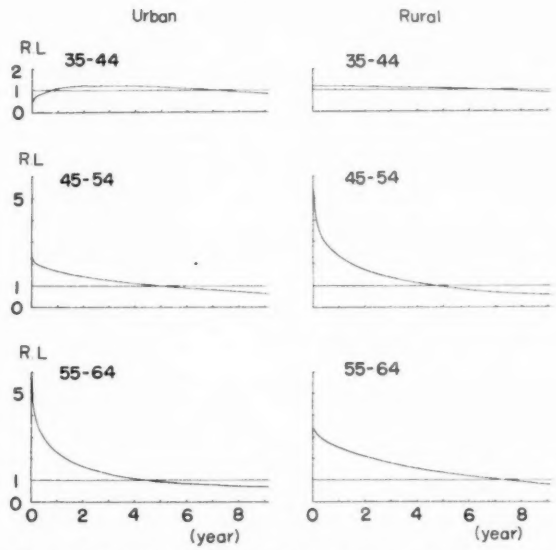


Fig. 2. Relative liability to breast cancer according to total lactation periods in ordinary measure.

In the urban 35-44 age-group, the s.r.m. mean concerning patients does not differ significantly from that concerning controls. When t.l.p. in ordinary measure is less than one year, the values of R.L. are less than unity. This means that the breast cancer grows at lower rate to the viviplara holding t.l.p. within one

year than to the whole vivipara (in the urban 35–44 age-group). Then R.L. remains around unity until the t.l.p. amounts to 10 years.

In the urban 45–54 age-group, the s.r.m. mean concerning patients differs significantly from that concerning controls. When t.l.p. (in ordinary measure) is less than one year, R.L. takes the values about 2. This means that the breast cancer grows nearly 2 times larger (in the rate) by the vivipara whose t.l.p. lies within one year compared with the whole vivipara. When t.l.p. increases to about 5 years, R.L. decreases monotonously to unity.

In the urban 55-64 age-group, the s.r.m. mean concerning patients differs significantly from that concerning controls. If t.l.p. vanishes, R.L. is equal to 6. When t.l.p. increases to one year, R.L. decreases rapidly to 2.3. R.L. vanishes at about 4 years of t.l.p..

In the urban district we may conclude that there is the close relation between the t.l.p. and the frequencies of breast cancer in the 45–64 age-group, but no relation in the 35–44 age-group.

In the rural 35–44 age-group, the s.r.m. mean concerning patients do not differ significantly from that concerning control. R.L. are almost equal to unity everywhere.

In the rural 45–54 age-group, the s.r.m. mean concerning patients differs significantly from that concerning controls. If t.l.p. vanishes, R.L. is equal to 5.4. When t.l.p. increases to one year, R.L. decreases rapidly to the value 2.4. R.L. takes the value 1.6 at 2 years of t.l.p.

In the rural 55–64 age-group, the s.r.m. mean concerning patients differs significantly from that concerning controls. If t.l.p. vanishes, R.L. is equal to 3.4 and less than the value 5.4 corresponding to the rural 45–54 age-group. But at the point of one year, R.L. takes the value 2.5 and is almost equal to that of the preceding age-group. When t.l.p. becomes to 2 years, R.L. is equal to 2.1 and greater than that of the preceding.

In the rural district, as in the urban, we may conclude that there is the close relation between the t.l.p. and the frequencies of breast cancer in the 45–64 age-group, but no relation in the 35–44 age-group.

SUMMARY

I have introduced the limiting function $l(x)$ of relative liability in the case where the populations are normal. It has become clear that the function $l(x)$ is instructive in the study on the quantitative relation of total lactation periods and frequencies of breast cancer.

We may safely conclude that in the urban and the rural district, the length of total lactation periods closely affects the frequencies of breast cancer in 45–64 age-group, though there is no particular relation between them in the 35–44 age-group.

In closing my report this time, I want to express my sincerest thanks to Professor Mitsuo Segi for the kind and thoughtful guidance extended to me.

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Tohoku J. Exper. Med., 1960, 72, 78-82

On a Rare Disease Due to Alternaria Tenuis Nees (Alternariasis)

By

Yasukuni Ohashi

Hanamaki Hospital (Director Takafusa Sato, M. D.)
Hanamaki City, Iwate Pref., Japan

(Received for publication, December 16, 1959)

From January, 1956 to June, 1958, the present author has experienced 37 cases of alternariasis due to a kind of fungi, the Alternaria tenuis Nees, but seeing that the fungus has been neglected as a non-pathogenic filamentous fungus by the medical circle and that reports on the disease cases due to it are only very limited in number in the western countries as well as Japan, the following clinical and experimental studies have been undertaken, to obtain some data on its details.

CLINICAL CASES

A boy of 11 years old with no remarkable anamnesia began to complain of pollakisuria, hematuria and urodynia and visited Hanamaki Hospital as an expatient. At the first examination, renal tuberculosis was suspected of, but upon various tests no anomaly was observed to substantiate the suspicion.

Upon repeated urine test, numerous spores of Alternaria tenuis Nees, brown colored, ellipsoid in form, $10 \times 5\,\mu$ in size, having a single septum at one pole,

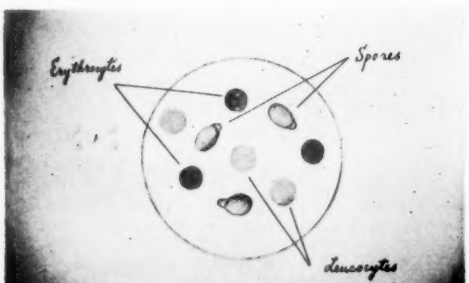

Fig. 1. Spores in the urine of a patient.

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were detected. (Fig. 1)

Since then similar spores were found in the cerebrospinal fluid, the gall or the sputum, of 37 patients with hematuria, meningitis, cholecystopathy etc. at Hanamaki Hospital within thirty months.

PURE CULTURE

Pure culture of these spores found in the urine of the patients was successfully carried out by using potato-glucose agar and incubating at around 25.0°C. The colonies became macroscopically visible in 1 or 3 days and grew to 2 or 3 cm in diameter in about 5 days; they were dark green in color and a white fringe encircled them. Microscopically examined, the spores were found to send out germ tubes from one or both of their poles in several hours after the beginning of the culture and in 60 hours or so, the conidia were perfected, which are ovoid in form, have several segments each and are 20–60 μ in length; several such conidia were found aligned in chains. (Fig. 2)

Fig. 2. Conidia formed on the tips of Conidiophores.

ANIMAL EXPERIMENT AND AUTOPSY FINDINGS

For determinating the toxicity of this fungus to animals, the following experiments were undertaken, using spores obtained by the pure culture above.

Method: 7 guinea-pigs were intraperitoneally injected with 1 cc each of physiological saline solution containing about 600,000 to 1,000,000 of spores of the fungus in suspension, and animals were examined as follows.

Results: 5 of the 7 guinea-pigs died in 8 days on the average after the injection. It must be noted that spores of the Alternaria was detected in urine of all the animals. The urination per day markedly decreased on the day of death and the previous day. The body weight decreased by 15–20 %.

Post-mortem findings: Spores were found in the lung, the liver, the kidney and the adrenal gland as well as in the gall and the urine drawn out by puncture

from the excised gall-bladder and urine bladder. Histologically examined, all these organs were prominently blood-congested and various inflammatory changes were observed in them.

THERAPEUTIC RESEARCHES

By tests with sensitivity tablets, the fungus was found unaffected in the growth by any antibiotics except by trichomycin (Fujisawa Pharmaceutical Co. Ltd., Japan) (Fig. 3).

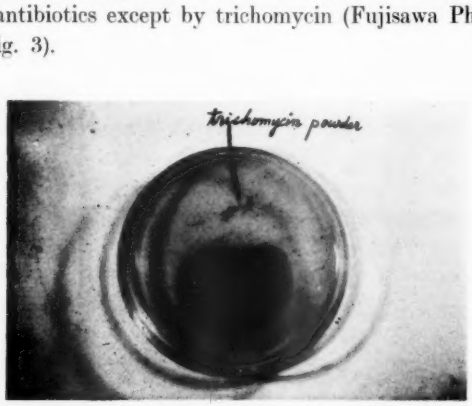

Fig. 3. Fungual growth inhibited by trichomycin powder.

On a medium containing only 30 units of trichomycin per cc, the growth of this fungus is totally inhibited. It was experimentally verified that when trichomycin had been orally administered to guinea-pigs before intraperitoneal injection of the spores, the animal survived. In clinical application too, I found several cases in whom only administration of trichomycin was effective in eliminating spores of the Alternaria from their urine. These findings indicate that trichomycin is most effective in treating alternariasis.

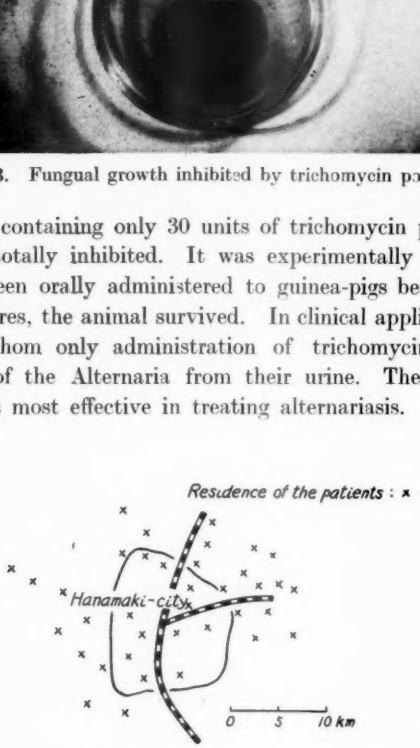

Fig. 4. Distribution of the 37 patients.

EPIDEMIOLOGICAL STUDY

The above-mentioned 37 cases of alternariasis were all found in an area within the distance of several miles from Hanamaki (Fig. 4) ,and spores of the pathogenic fungus were detected in the water from the wells used by some of the patients.

In one family using a well infested with such spores, 3 of its members were infected. From such findings, it may be concluded that this fungus is distributed widely in the soil in the district around Hanamaki and occasionally gets into wells to cause infection in some of the users of the wells.

ON PROPHYLAXIS

The best way to prevent infection through well water is disinfection by boiling the water for more than 10 minutes, for the fungus could be killed by boiling for not less than ten minutes only. Chlorinated lime and such chemicals often used for disinfecting well water are utterly ineffective against the fungus, so that the above boiling method is the only way of sure disinfection.

DISCUSSION

The Alternaria tenuis Nees was first described by Nees[1] in 1817 and Saccardo-Lindau[2] says that the fungus belongs in the order of Fungi imperfecta of the class of Eumycetes and is widely distributed in the flora, being sometimes detected in the soil and the atmosphere too. But very few works have reported on the pathogenity of this fungus and scarcely any clinical study had been conducted concerning the disease caused by it.

Only Matta[3] (1926), Hopkins[4] (1930), Castellani[5] (1926) and Borsook[6] (1933) reported on one case each of otopathy, asthma and vaginitis caused by the fungus and Wahl[7] reported on the detection of the fungus in the sputum of 3 cases of lung tuberculosis.

In Japan, Ohue[8] at Matsuyama High School alone, wrote on the fungus from the mycological point of view. There has never been any report on a mass outbreak of alternariasis as I found in the above 37 cases met with at Hanamaki and it may be possibly an endemic in the district, but I suspect that if more adequate attention is paid to the test of urine, patients of this disease may be found in other localities too.

SUMMARY

1) The present author has experienced 37 cases of alternariasis in and around Hanamaki City, Iwate Prefecture, between January, 1956 and June, 1958.

2) Pure culture was successfully carried out with spores of the fungus isolated from the urine of the patients, using potato-glucose agar medium.

3) Upon experimental infection of guinea-pig with the fungus, the death rate of ca. 70 % ensued with 8 days for existence on the average.

4) For treating the disease, trichomycin was found most efficacious.

5) Infection by the fungus seems to come orally through drinkng well water and the only sure way of prophylaxis is boiling water at least for 10 minutes before use.

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Tohoku J. Exper. Med., 1960, 72, 83-90

Role of the Peripheral Sensory Pathway in Performance of the Conditioned Leg Flexion Reflex

By

Chosaburo Yamamoto

From the Department of Physiology, University of Kanazawa Medical School, Kanazawa

(Received for publication, December 19, 1959)

It has been open to serious question as to whether sensory pathways for the unconditioned stimulus must be kept intact in order for a pre-established conditioned reflex to be performed. In 1923, Lang and Olmsted[1], working with dogs, found that a conditioned flexion reflex of the hind leg was abolished after hemisection of the spinal cord on the side opposite to the trained leg. These authors considered the abolition of the conditioned response as due to interruption of the intramedullary afferent pathway of pain. However, using the temporary cold block method to exclude the ascending pain pathways for an unconditioned stimulus, two groups of investigators[2),3)] could not confirm the conclusion reached by Lang and Olmsted. In 1939, the problem was again approached by Martino[4)] studying in dogs a conditioned blink reflex which was established by an unconditioned electric shock applied to the skin around the eye. He reported that the conditioned blink reflex so established could be abolished by anaesthetizing the locus of the skin which had served as the receiving zone of the unconditioned stimulus.

In the present experiment, aimed at studying the role of the sensory pathway for the unconditioned stimulus in the leg flexion reflex, special attention was paid to the following two points. (1) Selective interruption of the sensory pathway without giving any lesion to the motor system. For this purpose, an entire series of dorsal roots supplying the trained hind leg were completely sectioned. (2) Types of the conditioned response. According to the training technique employed, the conditioned response is classified in two types. One is the classical type and the other is the instrumental (cf. section of method). Effects of the deafferentation were studied on both types of conditioned reflex in comparison with each other. The results of the present investigation show that following the section of the dorsal roots, the classical type of conditioned response tends to be abolished while the instrumental type remains apparently unaffected.

山本　長三郎

METHOD

Adult dogs were used. They had never been subjected to any sort of conditioning before the present experiment.

Training procedure. Most of the experiments were conducted with dogs which were made to stand on a cradle, being fastend to it loosely with straps.

The conditioned stimulus used in the present experiment was a 1000 cps tone lasting for 5 seconds, and the unconditioned stimulus was an electric shock applied for about 1 second to the webs of the left hind leg through a pair of clipp electrodes. When the training was conducted according to the classical procedure, the animal was always shocked 4 seconds after the commencement of the conditioned stimulus. On the other hand, the procedure of instrumental conditioning adopted in the present experiment was such that the animal received the unconditioned shock stimulus only when it failed to show a conditioned leg flexion upon the conditioned stimulus with a latency less than 4 seconds. Thus, the difference between the classical and the instrumental conditioning was such that the classically trained dog received the electric shock invariably following the conditioned stimulus whereas the instrumentally conditioned one could avoid the shock by performing the conditioned response.

Applying the conditioned stimulus 12 times in one daily session, whether or not accompanied by the unconditioned stimulus, it was found in both types of trianing procedure that the conditioned flexion response of the left hind leg was established with a considerable stability within a few sessions.

Operation. Under pentobarbital sodium anaesthesia and aseptic conditions, the spines and laminae of the lumbar and sacral vertebrae were cut away. To expose the dorsal roots extradurally, the spinal cord was gently retracted on one side and fat tissues covering the roots were removed. Usually, a series of the dorsal roots were sectioned from L_3 to S_3. In some animals L_1 and L_2 dorsal roots were also removed.

Program of experiments. The method of evaluating effects of the deafferentation consists in comparing the percentage frequency of correct conditioned responses between pre- and postoperation. Here, the percentage frequency of correct conditioned responses is defined as a ratio of the number of correct conditioned responses to the number of times the conditioned stimulus was presented. However, there is a need for some comment concerning the preoperative score. The operation of deafferentation is so complete that the operated animal can not perceive the electric shock applied. This means that for the postoperative period, the conditioned repsonse is left unstrengthened by the unconditioned stimulus, whether or not the conditioned response is performed. Corresponding with this postoperative situation, we must have, prior to the operation, an observation period, during which the conditioned response is never

reinforced. The score referred to as preoperative is the one obtained during this observation period, and only this score can serve as control. After passing through a preoperative period for the control observation, the dogs were again subjected to the usual conditioning procedure for 1 to 2 weeks for the purpose of strengthening the conditioned response. Thereafter, the surgical operation was carried out. In regard to the program of the experiment, no distinction was made between the classical and the instrumental type of the conditioned response.

RESULTS

Postoperative motor behaviours

After the complete deafferentation the affected leg became atonic and could not support the weight of the body. The dog stood on the other three legs, slightly bending the anaesthetized leg at the knee and ankle joints with the dorsum of the foot resting on the floor. Being excited, however, the dog repeatedly flexed and extended the affected limb (Fig. 1). In some dogs there was

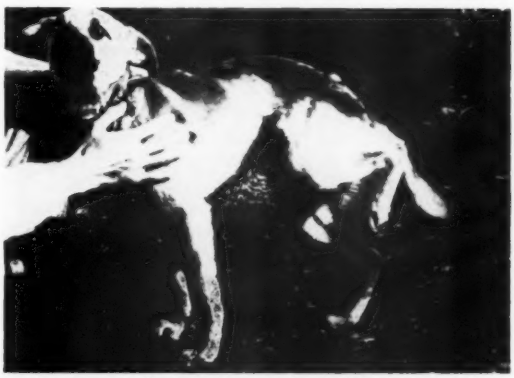

Fig. 1. Repeated flexions of anaesthetized limb. Dorsal roots were sectioned entirely from L_3 to S_3 on the left side 4 days previously.

observed an exaggerated scratch reflex in the affected leg. When the animal walked with the intact three legs, the affected leg showed flexion movements associated with stepping, though it was ataxic and ineffective in walking. In the course of about two weeks, a hyperextension of the operated leg developed in some dogs. In these cases the leg showed much stiffness and somewhat heightened resistance to a passive flexion, and it was dragged behind in walking. Effects of the complete deafferentation of a limb had been observed by Ranson[5] in cats and more recently by Twitchel[6] in monkeys. Except for details, the postoperative symptoms observed in our dogs were in good agreement with those reported by previous workers.

Classical conditioned response

The conditioned responses in a number of dogs trained according to the classical procedure has a tendency to weaken spontaneously. Throughout the preoperative observation period in which the conditioned response was never reinforced, most of the dogs showed scores too unstable to be used as control. Only three animals could be firmly conditioned and these were subjected to the deafferentation. The curve of Fig. 2 was obtained from one of the three dogs.

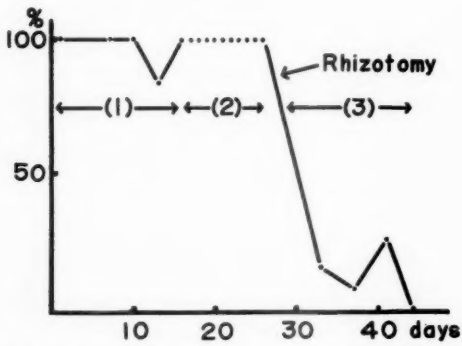

Fig. 2. Effects of deafferentation in a classically conditioned dog. Dorsal roots were removed from L_1 to S_3. Ordinate, percentage frequency of correct conditioned responses; abscissa, days after beginning of preoperative control observation. 1. Preoperative observation period; 2. retraining period; 3. postoperative observation period.

Throughout the preoperative observation period, this dog could maintain a high activity level of the conditioned response without the reinforcement. After this control curve was drawn, the conditioned response was reinforced for 10 days and then the dorsal roots were sectioned from L_1 to S_3 on the side of the trained leg. The postoperative curve shows how abruptly the scores of the conditioned response decreased. The latency of the conditioned response was prolonged and the amplitude was decreased, though occasionally beautiful conditioned responses were elicited. Except for minor details, results obtained from the remaining two dogs were found essentially similar to that shown in Fig. 2. Before the operation, we observed in one of these dogs that, while standing on a cradle, it repeatedly flexed the trained leg spontaneously in the interval of stimulus trial. This spontaneous flexion movement also disappeared almost completely after the operation. In all the dogs tested so far, generalized responses to the conditioned stimulus, such as growling, accelerated respiration and stepping of the forepaws, were occasionally seen, even if the conditioned flexion responses were completely lost sight of.

Instrumental conditioned reflex

In contrast with the classical conditioned response, the operation failed to bring about any noticeable effects upon the instrumental conditioned response. An example of the results is presented in Fig. 3, in which the percentage freqency

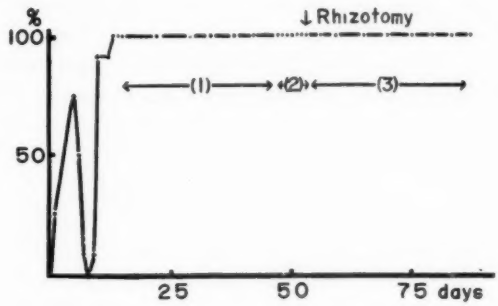

Fig. 3. The curve represents percentage frequency of correct conditioned responses of a dog trained according to instrumental training procedure. Dorsal rhizotomy was performed from L_3 to S_3. Abscissa indicates days after beginning of preoperative training. 1, 2, & 3, same as in Fig. 2.

of the correct conditioned responses is found to keep high values during the post-operative observation period as long as 40 days. Neither more extensive section of the dorsal roots including L_1 and L_2 nor prolongation of the observation period succeeded to detect any signs of lowered activity of the conditioned response. In some animals, in which a hyperextension developed in the operated hind leg, the conditioned activity was curiously modified in such a way that a strong extension, instead of a flexion, was elicited as a conditioned response at the knee and foot joints. In response to the conditioned stimulus, the leg which maintained a slightly flexed posture before the conditioned stimulus was started, was stretched and became so stiff that it appeared like a rigid pillar. After the extension lasting for 0.5-1 second the leg assumed its original posture of a slight flexion and this extension-flexion movement was repeated several times until the conditioned stimulus was turned off.

As a more sensitive indicator of the strength of conditioning, the degree of generalization to tones of various pitches was determined with one dog. The dog was trained with a 1000 cps tone as a positive conditioned stimulus. With this dog a 60 cps tone was made as a negative conditioned stimulus, i.e. the dog was punished whenever it responded to a 60 cps tone with a flexion of the left hind leg. After the differentiation between 1000 and 60 cps tones was firmly established, two sessions were devoted to measure to what extent the conditioned

response occurred to tones having intermediate frequencies between 1000 and 60 cps. In those sessions, 1000, 750, 500, 250 and 60 cps tones were delivered in a random order. The dog made the correct conditioned response to 1000 and 750 cps tones and never responded to 250 and 60 cps tones. To 500 cps tone, it showed the leg flexion in some trials and it did not in other trials. Thus, the conditioned response score took an intermediate value between 0 and 100 for 500 cps. One week after the operation in which the dorsal roots from L_3 to S_3 were sectioned, the degree of the generalization was again examined, using a 400 cps tone besides the series of tones as mentioned above. Fig. 4 illustrates

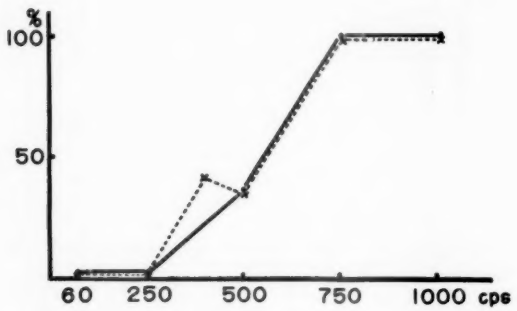

Fig. 4. Generalization curves in an instrumentally conditioned dog. The dog was trained to make a differentiation between 1000 cps tone (positive stimulus) and 60 cps tone (negative stimulus). Solid line, before operation; dotted line, after operation. Dorsal roots were sectioned from L_3 to S_3.

the generalization curve obtained before (solid line) and after (dotted line) the operation. The almost exact coincidence of the two curves may indicate that the operation was ineffective in changing the strength of conditioning as measured by the degree of generalization.

DISCUSSION

The strength of the classical conditioning decreased abruptly after the complete deafferentation of the trained limb. The participation of the afferent impulses in eliciting the conditioned response may occur at any level of the central nervous system. It is well recognized that the corticospinal neurones change their excitability after the arrival of specific or unspecific afferent volley.[7),8)] Magoun and his collaborators[9),10)] proved that the mesencephalic reticular formation which received sensory impulses through the afferent collaterals facilitated or inhibited the spinal reflex. Granit and his associates[11)] demonstrated that

some kinds of descending impulses could activate the spinal motoneurones by means of the spindle afferent system. Concerning the classical conditioning, we presume that the afferent volleys from the trained leg may play a main part in maintaining the excitability of the efferent system upon which the performance of the conditioned response is dependent. Then, the initiation of the descending impulses from the motor cortex and the activation of the spinal motoneurones may be suppressed after the deafferentation. As a final result, the classical conditioned response is expected to be abolished.

A nervous mechanism underlying the instrumental conditioned response must be thought entirely different from that of the classical one. In the instrumental training procedure the reinforcing stimulus is, as indicated by Hilgard and Marquis,[12] the lack of the unconditioned stimulus. The fact that only the training situation is sufficient to maintain the conditioned response for a long period, suggests that the activity level of the efferent system is supported not by the afferent impulses coming from the trained limb but by those arising from the receptors for the experimental situation. The results of the present experiments lend considerable support to this conjecture.

In the present experiment, it was established that interruption of the sensory pathway for the unconditioned stimulus was effective in abolishing the conditioned responses only when the classically trained dogs were used as subjects. Lang and Olmsted,[1] and Martino[4] who reported the abolition of the conditioned responses after anaesthetization of the receptive field of the unconditioned stimulus, adopted the classical conditioning procedure, and in this regard their results were in general accord with ours.

SUMMARY

Effects of the posterior rhizotomy were studied on the conditioned leg flexion responses in dogs, paying special attention to whether the conditioned response was established by the classical procedure or by the instrumental one. The following results were obtained.

1. The classical conditioned response lost its preoperative high scores after the dorsal roots supplying the trained leg were completely sectioned.

2. The instrumental conditioned response suffered no detectable changes after the posterior rhizotomy.

3. A discussion was made concerning the role of afferent impulses in maintaining activity of the conditioned response.

The author is grateful to Dr. Kitsuya Iwama for his helpful discussion and direction during the initiation and course of this research. A part of the expense of this work was defrayed by a grant of the Ministry of Education awarded to Dr. Iwama.

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The Age-adjusted Death Rates for Malignant Neoplasms in Some Selected Sites in 23 Countries in 1954-1955 and their Geographical Correlation

By

Mitsuo Segi, Susumu Fujisaku, Minoru Kurihara, Yoko Narai and **Kyoko Sasajima**

From the Department of Public Health, Tohoku University School of Medicine, Sendai; Director: Prof. M. Segi

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We cannot deny that, with the recent rise in the recovery rate of cancers, the significance of mortality statistics in studying the geographical distribution of cancer has been reduced, but seeing that in many part of the world, however, no survey on cancer morbidity has been carried out to date, we believe, the significance has not yet been lost altogether. We applied to the central statistical administrations of many countries and have been kindly supplied with data on the mortality for cancer. In this report, we will write on the age-adjusted death rates for cancer for the two years of 1954 and 1955 and on the geographical correlation among them.

1. Method of Study

We have been furnished with the statistical data on the population and the mortality for cancers in some specified sites by the central statistical administrations of 23 countries and calculated the age-adjusted death rates. The countries from which we were furnished the data were as follows:

Union of South Africa, Chile, Canada, United States, Israel, Japan, Germany, Austria, Belgium, Denmark, Finland, France, Ireland, Italy, Norway, Netherlands, England and Wales, Scotland, Northern Ireland, Sweden, Switzerland, Australia and New Zealand

The data from U.S.A. were given separately for whites and non-whites. Of the Union of South Africa, we could not obtain the data on the population as itemized by age-classes for 1954 or 1955, and we had to make our computation based on the figures for 1952 and 1953. Besides, we received from Portugal the data only for 1955, but not for 1954, so we have excluded them from our present study.

The data furnished consisted of the numbers of deaths for cancers in the

瀬木三雄，藤咲　遥，栗原　登，奈良井容子，笹島鏡子

following sites, itemized into five-yearly age-classes.

All sites (Int. Detailed List No. 140–205), Esophagus (150), Stomach (151), Intestine, except rectum (152 and 153), Rectum (154), Pancreas (157), Trachea, bronchus and lung (162 and 163), Breast (170), Uterus (171, 172, 173 and 174), Ovary, Fallopian tube, and broad ligament (175), Prostate (177) and Leukemia and aleukemia (204)

For computing the adjusted death rates, we took the total male and female populations of the 46 countries cited in the Annual Epidemiological and Vital Statistics 1950, 1951, 1952 and 1953[1),2),3),4)], as standard. The names of the countries cited and the years of the census are as follows:

1948: Philippines

1950: Australia, Belgium, Bolivia, Brazil, Ceylon, Denmark, Dominican Republic, East Berlin, Ecuador, El Salvador, France, Finland, Germany Fed. Rep., Guatemala, Honduras, Israel, Japan, Mexico, Netherlands, Nicaragua, Norway, Paraguay, Portugal, Puerto Rico, Sweden, Switzerland, Taiwan (China), Turkey, United States, Venezuela, West Berlin

1951: Austria, Canada, England and Wales, Greece, Ireland Republic, Italy, Jamaica, New Zealand, Northern Ireland, Saar, Scotland, Thailand, Union of South Africa

1953: Yugoslavia

In actual computation, we used the five-yearly age-class populations per 100,000 total population, but the age-classes below 20 and above 75 were treated

TABLE I. Total Male and Female Population and Composition per 100,000 Population by Five-year Age-classes of 46 Countries

Age	Population	Composition per 100,000 population
All ages	714,535,109	100,000
0 – 4	83,037,168	39,233
5 – 9	70,460,703	
10 – 14	65,520,913	
15 – 19	61,200,136	
20 – 24	59,486,896	8,329
25 – 29	55,787,630	7,811
30 – 34	45,977,472	6,437
35 – 39	48,496,194	6,790
40 – 44	45,064,637	6,309
45 – 49	40,552,636	5,678
50 – 54	35,193,114	4,927
55 – 59	28,682,340	4,016
60 – 64	24,882,338	3,484
65 – 69	19,737,396	2,763
70 – 74	14,571,567	2,040
75+	15,590,875	2,183
Unknown	293,094	—

Refer to 1. Method of Study above for the names of the countries and the years of census.

as single classes respectively. The populations used as standard and their distribution per 1000,000 total populace are as shown in Table I.

The adjusted male and female death rates for cancers of all sites and the above specified sites of the countries cited are as shown in Table II.

2. Observations on the Age-adjusted Death Rates for Cancers in 23 Countries

The adjusted death rates for all the items 140–205 in the three-digit categories of the Sixth Revision of International Statistical Classification of Disease, Injuries and Causes of Death, as computed by the method outlined above, are shown in Table II.

The male death rate is the highest in Finland, followed by Scotland, Austria, England and Wales and Switzerland. The rate is the lowest in Israel, standing at about 60% of that in Finland. The female rate is the highest in Denmark, then in Chile and the lowest in Japan, being about 65% of that in Denmark.

As shown in Table II, the age-adjusted death rate for esophagal cancer among males is particularly high in Switzerland, followed by France, Chile and Finland and the lowest in Sweden, showing a rate of about one-fifth of the highest

TABLE II. Age-adjusted Death Rates for Malignant Neoplasms in Selected Sites by Sex in 23 Countries, 1954 and 1955
(Rates per 100,000 population. Rates were standardized on the basis of the age distribution of the total sum of the populations of male and female in 46 countries.)

	140—205 All sites		150 Esophagus		151 Stomach		152, 153 Intestine	
	Male	Female	Male	Female	Male	Female	Male	Female
South Africa†	149.5	125.1	4.0	1.2	35.4	20.9	10.0	10.5
Chile	138.0	134.9	8.1	4.1	69.7	47.6	2.3	3.4
Canada	131.8	114.9	2.9	1.2	23.5	12.1	13.1	16.0
U.S., White	136.2	112.5	3.3	0.8	14.9	7.7	12.9	13.8
U.S., Nonwhite	140.2	123.3	6.8	1.8	23.5	10.5	9.4	9.8
Israel	114.8	119.3	4.3	2.3	23.1	17.5	·	·
Japan	121.8	92.6	6.7	2.2	68.3	36.6	2.3	2.5
Germany, Fed. Rep.	150.7	126.6	3.9	1.1	43.8	27.2	6.5	5.6
Austria	176.4	129.4	4.9	1.0	47.2	28.0	11.4	9.5
Belgium	149.2	122.4	3.7	1.4	33.2	20.0	10.7	11.4
Denmark	149.0	141.6	3.7	1.5	32.1	20.9	12.6	13.0
Finland	184.9	114.3	8.1	5.6	61.1	34.7	4.7	5.3
France	144.8	104.0	10.0	1.1	27.0	14.5	10.6	11.0
Ireland	129.7	110.1	4.4	3.5	27.8	20.0	13.2	13.6
Italy	119.1	95.2	3.7	0.9	35.1	20.7	5.4	5.5
Norway	124.1	110.8	3.5	1.0	38.0	22.0	7.6	6.9
Netherlands	147.0	124.3	3.4	1.5	36.3	22.8	9.6	10.9
England and Wales	168.5	114.5	4.6	2.3	27.5	15.2	14.2	13.8
Scotland	181.0	129.2	5.8	3.7	30.3	19.4	20.1	18.6
Northern Ireland	134.9	116.2	4.0	3.3	26.5	17.5	12.6	14.6
Sweden	115.4	109.7	2.6	1.4	30.8	19.5	9.2	8.2
Switzerland	166.6	119.5	13.1	1.5	39.0	23.2	11.3	9.3
Australia	127.1	101.2	3.6	1.1	22.7	12.2	13.0	14.2
New Zealand	134.1	111.5	3.6	2.3	20.3	13.2	14.2	15.3

TABLE II — Continued

	154 Rectum		157 Pancreas		162, 163 Trachea, bronchus and lung		170 Breast	171–174 Uterus
	Male	Female	Male	Female	Male	Female	Female	Female
South Africa†	5.2	4.0	6.0	4.7	23.0	5.8	21.9	16.5
Chile	1.8	1.9	·	·	11.2	4.7	8.3	20.2
Canada	7.9	4.5	7.0	4.5	19.4	3.8	22.2	13.5
U.S., White	6.8	4.6	7.1	4.4	24.0	4.0	21.7	14.0
U.S., Nonwhite	5.4	4.5	7.4	4.4	20.3	4.1	19.1	31.8
Israel	5.7	3.4	·	·	16.5	5.1	21.0	7.0
Japan	4.2	3.2	1.7	1.2	5.2	1.9	3.9	19.2
Germany, Fed. Rep.	7.7	4.5	3.6	2.4	25.2	4.0	15.1	13.9
Austria	9.1	4.8	5.7	3.9	40.0	5.2	14.8	21.0
Belgium	9.9	6.2	3.3	2.4	25.9	3.6	19.0	14.9
Denmark	12.3	6.6	6.1	4.1	19.6	4.3	24.2	17.8
Finland	4.5	3.7	4.3	3.1	44.8	4.3	11.7	11.4
France	6.7	3.8	·	·	15.6	3.3	14.0	12.6
Ireland	8.6	4.3	5.5	3.5	18.3	5.6	18.4	9.1
Italy	3.8	2.8	2.6	1.6	13.8	3.1	13.4	13.7
Norway	4.9	3.2	4.9	3.3	8.2	2.4	17.9	10.7
Netherlands	7.6	5.0	4.0	2.9	30.0	3.0	22.7	11.1
England and Wales	11.3	6.1	6.3	3.9	50.5	6.5	23.2	10.7
Scotland	10.7	5.5	6.3	4.4	50.3	7.5	22.5	13.1
Northern Ireland	9.2	4.9	5.1	4.0	28.5	4.9	18.8	10.1
Sweden	6.3	3.8	6.0	4.2	10.3	3.5	18.9	10.9
Switzerland	9.1	4.7	4.6	3.4	26.2	3.0	22.2	13.2
Australia	6.3	3.8	5.8	3.7	19.4	2.9	20.1	10.8
New Zealand	7.1	4.5	6.4	4.8	23.8	2.7	22.3	11.2

	175 Ovary, etc.*	177 Prostate	204 Leukemia and aleukemia	
	Female	Male	Male	Female
South Africa†	6.5	15.9	4.9	4.0
Chile	·	6.6	2.5	1.8
Canada	6.8	12.7	5.8	4.3
U.S., White	7.3	13.9	7.3	5.1
U.S., Nonwhite	5.3	20.6	4.9	3.0
Israel	·	6.3	6.5	6.1
Japan	1.2	0.8	2.7	1.8
Germany, Fed. Rep.	·	10.8	5.3	3.6
Austria	10.2	11.2	6.0	3.8
Belgium	4.7	12.6	4.8	4.0
Denmark	9.8	13.0	7.0	5.4
Finland	4.4	10.3	5.5	3.3
France	·	11.0	5.5	4.0
Ireland	4.2	8.7	·	·
Italy	2.4	6.6	4.7	3.5
Norway	8.0	13.9	6.1	5.2
Netherlands	7.6	12.3	5.5	4.3
England and Wales	7.7	11.4	4.9	3.5
Scotland	7.0	10.7	5.4	3.3
Northern Ireland	5.9	10.6	4.4	2.9
Sweden	8.0	12.5	6.2	4.8
Switzerland	7.9	14.0	5.5	4.0
Australia	5.6	15.2	5.0	3.8
New Zealand	6.0	15.1	6.3	5.2

* Including ovary, Fallopian tube, and broad ligament.

† The figures for South Africa are for 1952 and 1953.

rate shown by Switzerland. The female death rate in Finland is the highest, those in Chile, Scotland, Ireland and Northern Ireland coming next in descending order. The lowest rate is that in U.S.A., White—about one-seventh of that of Finland.

As shown in Table II, the age-adjusted death rate for stomach cancer is particularly high in Chile, Japan and Finland and the lowest among the U.S., Whites, both among males and females. The rate among the U.S., Whites is about one-fifth among males and about one-sixth among females of the rate in Chile.

The adjusted death rate for cancer of the intestine except the rectum is particularly high among both males and females in Scotland and low in Chile and Japan. The lowest male rate found in Chile is about one-ninth of that of the highest in Scotland and the lowest female rate shown by Japan is about one-seventh of that in Scotland.

The adjusted death rate for cancer of the rectum is the highest in Denmark among males, descending in the order of England and Wales, Scotland, Belgium and so forth down to Chile, the lowest.

The rate among females is also the highest in Denmark, followed by Belgium and England and Wales. Chile again stands at the tail-end.

The rates in Chile is about one-seventh among males and about one-fourth among females of the corresponding rates in Denmark.

The age-adjusted death rate for pancreatic cancer among males is the highest among the U.S., Non-whites, followed by U.S., Whites and Canada, Japan coming last in the list. Among females, the rate is the highest in New Zealand and the lowest in Japan. The death rate in Japan for cancer of this site is about one-fourth of that for males among the U.S., Non-whites as well as that for females in New Zealand, respectively.

The rate for cancer of the lung, including that of the trachea and the bronchus (Int. List Nos. 162 and 163), as shown in Table II is the highest among males in England and Wales, showing a markedly high level seconded closely by Scotland. Finland and Austria also show particularly high rates. The lowest rate, about one-tenth of that in England and Wales, is found in Japan. Among females, the rate is the highest in Scotland and next in England and Wales. The lowest rate in Japan is about one-fourth of that in Scotland.

The age-adjusted death rate for breast cancer is particularly high in Denmark, England and Wales etc., and particularly low in Japan, where it is only about one-sixth of that in Denmark. Chile and Finland also show low rates.

The age-adjusted death rate for cancers in all the parts of the uterus (Int. List Nos. 171 through 174) is very exceptionally high among U.S., Non-whites, standing 50% higher than the runner-up Austria. The rate is the lowest and comes up only to about one-fifth of that among the U.S., Non-whites in Israel.

We wished to calculate the age-adjusted death rates separately for cancers of the cervix and the corpus uteri respectively, but the data were regrettably insufficient for that, the deaths for cancer of the corpus uteri being unspecified in the statistics of some countries, while in those of Japan and many other countries, many cases of death for cancer of the corpus uteri are seemingly entered under the heading "cancer of uterus, unspecified (Int. List No. 174)".

The age-adjusted death rate for cancer of the ovary, including the Fallopian tube and the broad ligament (Int. List No. 175) is the highest in Austria and next in Denmark, and low in Japan and Italy. The rate in Japan, the lowest, is around one-ninth of that in Austria, the highest.

The age-adjusted death rate for cancer of the prostate is especially high among the U.S., Non-whites but exceptionally low in Japan. The rate in Japan is nearly one-twenty-fourth of that among U.S., Non-whites. The rate in Japan is only about one-eighth even of that of Israel just above her at the bottom of the list.

The age-adjusted death rate for leukemia and aleukemia, as shown in Table II is the highest among the U.S., Whites, next in Denmark and the lowest in Chile and next in Japan. The rate in Chile is about one-third of that among the U.S., Whites. Among females, the highest rate is found in Israel and the lowest in Japan and Chile. The rate in Japan is about one-third of that in Israel.

3. Sex Ratio of Age-adjusted Death Rates for Cancer

We next computed the sex ratios of the adjusted death rates for cancers in the sites considered above, excepting the cases where the cancer is not common to both sexes, in the form of male death rate/female death rate $\times$ 100. The results are shown in Table III.

The sex ratio of male to female death rates for cancers of all sites is the largest in Finland (162). England and Wales (147) and Scotland (140) come next, while in Israel (96) it is the lowest of all the 23 countries. Except in Israel, the death rate for cancer is higher in males than in females.

The sex ratio for esophagal cancer is the largest in France (927) and in Switzerland (860), indicating a very notable difference between the death rates of males and females. The ratio was the smallest in Northern Ireland (119) and next in Ireland (126), where the difference between the male and the female death rates is small. It is particularly notable here that the sex ratio for esophagal cancer shows such a wide inequality by countries.

The sex ratio for stomach cancer is the highest in U.S., Non-whites (223), next in Canada (194) and U.S., Whites (193). The smallest ratios were found in Israel (132) and Ireland (140).

The sex ratio for intestinal cancer is the highest in Switzerland (121), Austria (119) and Germany (115) and the lowest in Chile (68), Canada (82) and Northern

TABLE III. Sex ratios of Age-adjusted Death Rates for Malignant Neoplasms in Selected Sites in 23 Countries, 1954 and 1955

(The ratios are shown in the values $\frac{\text{male death rate}}{\text{female death rate}} \times 100$.

For method of age-adjusting, see the note at head of Table II.)

	140–205 All sites	150 Eso-phagus	151 Sto-mach	152, 153 Intes-tine	154 Rectum	157 Panc-reas	162, 163 Trachea, bronchus and lung	204 Leukemia and aleukemia
South Africa*	119	345	170	95	131	128	398	122
Chile	103	196	146	68	93	·	237	142
Canada	115	238	194	82	177	155	516	135
U.S., White	121	418	193	93	148	163	605	144
U.S., Nonwhite	114	383	223	96	119	170	500	165
Israel	96	192	132	·	166	·	326	106
Japan	132	299	187	93	133	143	271	153
Germany, Fed. Rep.	119	346	161	115	171	150	624	148
Austria	136	503	169	119	190	148	773	159
Belgium	122	273	166	93	160	135	727	121
Denmark	105	252	154	97	185	148	460	130
Finland	162	144	176	89	122	141	1,051	166
France	139	927	186	96	180	·	476	138
Ireland	118	126	140	98	203	156	325	·
Italy	125	421	170	99	134	160	448	136
Norway	112	363	172	110	151	149	336	119
Netherlands	118	226	159	88	152	138	996	128
England and Wales	147	200	181	103	186	161	777	137
Scotland	140	157	156	108	196	143	670	163
Northern Ireland	116	119	151	86	189	128	585	152
Sweden	105	193	158	112	164	144	299	128
Switzerland	139	860	168	121	196	134	873	136
Australia	126	325	186	91	169	159	661	131
New Zealand	120	159	154	93	156	133	891	120

* The figures for South Africa are for 1952 and 1953.

Ireland (86). The countries showing the ratio of above 100 counted 7 in total, namely, Switzerland, Austria, Germany, Sweden, Norway, Scotland and England and Wales. Here we must note that the difference between the male and the female death rates is rather small in any country cited and that in many countries the male death rate is lower than the female death rate.

The sex ratio for rectal cancer is the highest in Ireland (203), followed by Scotland (196), Switzerland (196) and Austria (190). The country showing the lowest ratio is Chile (93), the only country where the death rate for females is higher than that for males.

The sex ratio for pancreatic cancer was found high in U.S., Non-whites (170), U.S., Whites (163) and in England and Wales (161) and the lowest in the Union of South Africa and Northern Ireland (128 in both). It may be pointed out that the sex ratios of all the countries show small difference among them.

The sex ratio for lung cancer stood high in Finland (1,051), Netherlands (996), New Zealand (891), Switzerland (873), but lower in Chile (237), Japan (271) and Sweden (299). We may note that the sex ratio is high in all countries

and that it fluctuates over a wide range by countries.

The sex ratio of the adjusted death rates for leukemia and aleukemia is the highest in Finland (166), closely followed by U.S., Non-whites (165) and Soctland (163). The lowest rate was shown by Israel (106), indicating a notable similarity of the death rates of males and females. The sex ratio shows small difference by countries in this disease as well as in pancreatic and intestinal cancers.

4. Geographical Correlation between Age-adjusted Death Rates for Cancers in Different Sites in the Same Sex

The death rates for cancer of any specified site are found rising in some countries and falling in other. So a study of the geographical correlation between the death rates for cancer in different sites may be of little lasting significance, but it may be yet of some limited value.

A. Among Males

We computed the mutual correlation coefficients between adjusted death rates for cancers of the different sites of the esophagus, the stomach, the intestine, the rectum, the pancreas, the lung and the prostate and for leukemia among males in the cited countries and studied whether the population correlation coefficient falls to 0 or not.

The death rates for cancer of some sites were found strikingly high or exceptionally low in some countries. Thus, it may be sometimes of little meaning to compute the correlation coefficients by such divergent data and discuss their significance, but we proceeded to carry out the computation without rejecting such exceptionally high or low figures, and the results were as shown in Table IV.

We examined from these computed correlation coefficients the applicability of null hypothesis that requires the value of 0 for the population correlation coeffecient. As the result we established at what level of percentage of the former coefficient the hypothesis has to be rejected and indicate hereunder the values of the level of significance in parentheses.

No significant correlation was found between the death rate for esophagal cancer and that for cancer in any other site cited above in male, if we take 5% as the level of significance.

The death rate for stomach cancer showed negative correlation with that for intestinal cancer, pancreatic cancer, prostatic cancer, leukemia and rectal cancer, each at the significance level of 5%.

The death rate for intestinal cancer shows negative correlation with that for stomach cancer at the level of significance of 0.1%, as above, but positive correlation with that for rectal cancer, pancreatic cancer, leukemia, lung cancer and prostatic cancer respectively at the level of significance of 5%.

The death rate for cancer of the rectum showed a negative correlation to that of stomach cancer and positive correlation to the rates for intestinal cancer

TABLE IV. Correlation Coefficients between the Age-adjusted Death Rates for Malignant Neoplasms in Some Selected Sites among Males in 23 Countries and Corresponding Probabilities

	Esophagus	Stomach	Intestine	Rectum	Pancreas	Trachea, bronchus and lung	Prostate	Leukemia and aleukemia
Esophagus		+.396	−.214	−.129	−.176	+.076	−.099	−.308
Stomach	P>.05		−.764	−.432	−.713	−.073	−.562	−.638
Intestine	*P>.05	*.001>P		+.758	+.697	+.506	+.462	+.537
Rectum	*P>.05	*. 05>P>.025	.001>P		+.302	+.554	+.244	+.413
Pancreas	*P>.05	*.001>P	.001>P	P>.05		+.245	+.714	+.602
Trachea, bronchus and lung	P>.05	*P>.05	.02>P>.01	.005>P>.001	P>.05		+.189	+.168
Prostate	*P>.05	*.005>P>.001	.05>P>.025	P>.05	.001>P	P>.05		+.472
Leukemia and aleukemia	*P>.05	*.005>P>.001	.01>P>.005	P>.05	.005>P>.001	P>.05	.025>P>.02	

The mark * before the figures indicates negative correlation.

and the lung cancer, at the level of significance of 5%.

Between the death rates for pancreatic cancer and stomach cancer the correlation is negative at the significance level of 0.1% but the former stands in positive correlation with that for intestinal cancer, prostatic cancer and leukemia at the significance level of 5%.

The death rate for cancer of the lung shows positive correlation with that for rectal cancer and intestinal cancer at the significance level of 5%.

The death rate for cancer of the prostate is in negative correlation with that for stomach cancer at the significance level of 0.5% but in positive correlation with that for pancreatic cancer, leukemia and intestinal cancer at the level of significance of 5%.

The adjusted death rate for leukemia showed negative correlation with that for stomach cancer at 0.5% significance level, but positive correlation with that for pancreatic cancer, intestinal cancer and prostatic cancer, at the level of significance of 5%.

B. Among Females

We made similar studies on the geographical correlation between the age-adjusted death rates among females for cancers of the following sites: The esophagus, the stomach, the intestine, the rectum, the pancreas, the lung, the breast, the uterus, the ovary and leukemia and aleukemia. The method of tests was the same as in the preceding study on male death-rates. The results were as shown in Table V.

The death rate for esophagal cancer among females stood in positive correlation with that for stomach cancer and lung cancer but in negative correlation with that for leukemia, at the level of significance of 5%.

The death rate for stomach cancer shows a positive correlation with that for esophagal cancer at the significance level of 2%, but with that for intestinal cancer, mammary cancer, pancreatic cancer, leukemia and rectal cancer the correlation was always negative at the level of significance of 5%.

Between the death rate for intestinal cancer and that for gastric cancer a negative correlation was established at the significance level of 0.1%, as above, but positive correlation was observed between the former and that for pancreatic cancer, cancer of the breast, rectal cancer, intestinal cancer, pulmonary cancer and leukemia and aleukemia at the significance level of 5%.

The death rate for rectal cancer stood in positive correlation with that for mammary cancer, intestinal cancer and ovarian cancer but in negative correlation with that for stomach cancer, at the significance level of 5%.

The death rate for pancreatic cancer is negatively correlated with that for gastric cancer at the significance level of 0.5%, but positively correlated with that for intestinal cancer, mammary cancer, ovarian cancer, lung cancer and leukemia at the significance level of 5%.

TABLE V. Correlation Coefficients between the Age-adjusted Death Rates for Malignant Neoplasms in Some Selected Sites among Females in 23 Countries and Corresponding Probabilities

	Esophagus	Stomach	Intestine	Rectum	Pancreas	Trachea, bronchus and lung	Breast	Uterus	Ovary etc.	Leukemia and aleukemia
Esophagus		+.481	−.065	−.140	+.032	+.405	−.287	−.112	−.303	−.429
Stomach	.02>P>.01		−.741	−.431	−.644	−.026	−.729	+.182	−.243	−.562
Intestine	*P>.05	*.001>P		+.633	+.730	+.422	+.796	−.296	+.386	+.439
Rectum	*P>.05	*.05>P>.025	.005>P>.001		+.346	+.326	+.654	−.002	+.514	+.270
Pancreas	P>.05	*.005>P>.001	.001>P	P>.05		+.454	+.738	+.036	+.629	+.485
Trachea, bronchus and lung	.05>P>.025	P>.05	.05>P>.025	P>.05	.05>P>.025		+.292	−.024	+.281	−.094
Breast	*P>.05	*.001>P	.001>P	.001>P	.001>P	P>.05		−.278	+.655	+.669
Uterus	*P>.05	P>.05	*P>.05	*P>.05	P>.05	*P>.05	*P>.05		−.021	−.467
Ovary etc.	*P>.05	*P>.05	P>.05	.025>P>.02	.005>P>.001	P>.05	.005>P>.001	*P>.05		+.637
Leukemia and aleukemia	*.05>P>.025	*.01>P>.005	.05>P>.025	P>.05	.05>P>.025	*P>.05	.001>P	*.025>P>.02	.005>P>.001	

The mark * before the figures indicates negative correlation.

The death rate for lung cancer shows positive correlation with that for esophagal cancer, intestinal cancer and pancreatic cancer at the significance level of 5%.

The same for mammary cancer stands in negative correlation with that for stomach cancer at the level of significance of 0.1%, but in positive correlation with that for intestine cancer, rectal cancer, pancreatic cancer, leukemia and ovarian cancer at the significance level of 5%.

The death rate for cancer of the uterus shows negative correlation with that for leukemia at the significance level of 2.5%, but no significant correlation was found between the death rate for uterine cancer and that for cancer of any other site studied here at the significance level of 5%.

The death rate for ovarian cancer stands in positive correlation with that for pancreatic cancer, mammarian cancer, leukemia and rectal cancer at the significance level of 5%.

The death rate for leukemia shows negative correlation with that for stomach cancer, uterine cancer and esophagal cancer and positive correlation with that for mammary cancer, ovarian cancer, intestinal cancer and pancreatic cancer, at the significance level of 5% each.

5. Geographical Correlation of Age-adjusted Death Rates for Cancer of the Same Site by Sex

In the last place, we made a statistical review on the question whether the death rate for cancer in all sites or in a certain site among females is high when the corresponding rate in the same country is high among males. The following correlation coefficients were obtained.

All sites	+0.494	Rectum	+0.930
Esophagus	+0.299	Pancreas	+0.950
Stomach	+0.968	Trachea, bronchus and lung	+0.638
Intestine	+0.960	Leukemia and aleukemia	+0.892

With these correlation coefficients, the null hypothesis (population correlation coefficient = 0) was tried and we found that the death rates for gastric, intestinal, rectal, pancreatic and lung cancers at the level of significance of 0.1% and that for cancers in all sites at the level of significance of 2% showed positive correlation. But no significant correlation could be found in the case of esophagal cancer, that is, the parallelism of the male and female death rates was not apparent in the case of esophagal cancer alone. This seems to suggest a clue in the search for the pathogenic cause of cancer of the esophagus.

We also studied whether these were any correlation between the death rate for prostatic cancer and that for female mammary cancer and between the former and that for ovarian cancer. The correlation coefficients were computed as +0.656 and +0.512 respectively, showing positive correlations at the levels of

significance of 0.1% and 0.25%, respectively.

SUMMARY

Basing ourselves on the population and mortality statistics furnished us by the central statistical administrations of 23 countries, we computed the age-adjusted death rates for cancers of all sites, the esophagus, the stomach, the intestine, the rectum, the pancreas, the lung, the breast, the uterus, the ovary and the prostate and of leukemia and aleukemia in these countries in the years 1954–55, taking the sum of the male and female populations by five-year age-classes of 46 countries around 1950 as standard, for examining the status of cancer mortality in these countries. The items surveyed comprise comparisons of the adjusted death-rates by countries and by sites, comparison of the sex ratios of the death rates by sites and by countries, the geographical correlation of male and female death rates by sites and the geographical correlation of male and female death rates of cancers of the same sites.

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The Effect of Phosphorus-Organic Compounds on Ascites Tumor in Mice

By

Hans Siering

University Hospital Medical Department Jena, Germany;
Director: Prof. Dr. W. Brednow

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Tri-alkyl-phosphines including their derivatives are phosphorus-organic compounds deriving from phosphorus-hydrogen. In their composition and properties they largely correspond to the amines. They are, however, slightly basic and easily inflammatory. Up to the present the phosphines have hardly been employed in experiment and therapeutics. Special importance must be attributed to their capability to form addition compounds with acids. In the event of such additions ensuing with nucleic acid, a proliferation suppressing effect is to be expected. It was for that reason that several phosphine derivatives were investigated with regard to their influence on the growth of ascites-carcinoma in mice.

In a number of mice, which have been injected intraperitoneally every other day with 250 gamma triethyl-phosphine dibromide, the daily increase in weight per mouse amounted to 0.07 g, while in the case of mice not treated in this way the figure was 0.8 g. The series of mice treated as above survived the non-treated specimens twice the time as from the date of the first injection (Fig. 1) When injected daily, an tumor inhibitive effect was observed, the injection amounting to 20 up to 100 gamma per mouse, the weight of the mice ranging between 20 to 24 g. When giving smaller doses the diminution of the growth of the inoculated tumor was unimportant.

An inhibition of the development of the tumor could also be achieved after the administration of other tri-alkyl-phosphine dihalogenides (triethyl-phosphine dichloride, tri-propyl-phosphine dibromide, and tributyl-phosphine dibromide).

A confirmation of our hypothesis that, due to its chemical characteristics, the phosphine molecule itself has a cytostatic effect, has been proved by the successful treatment with tri-ethyl-phosphine oxide. Also in this case, the development of the tumor was evidently slowed down and life of these mice obviously prolonged. The phosphine molecule itself has an important influence on the number of the mitoses and their specification.

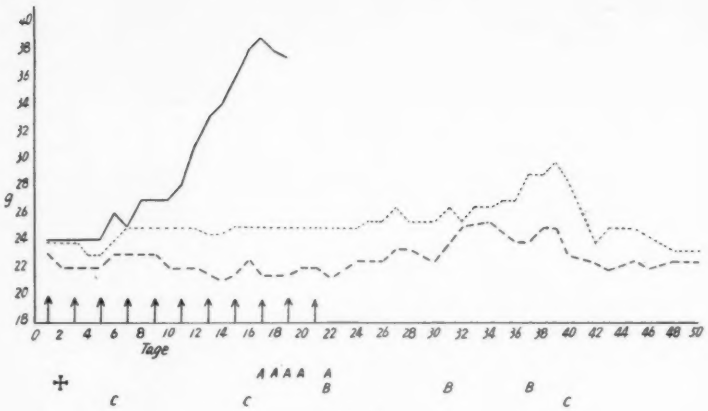

Fig. 1.

A —— Ascites 0.3/1 : 3
B ----- " " +250 γ Triethylphosphine dibromide every other day i.p.
C ····· 250 γ Triethylphosphine dibromide every other day i.p.

With the phosphines or its derivatives a new group of chemical compounds, not as yet mentioned in medical literature, is gaining importance. Apart from some occasional, halogen conditioned, local stimulating effects, toxic by-effects have not been detected after treatment with these substances. Regarding the cytostatic effect, the alkyl-groups are without any importance, since they are so rigidly combined with the phosphorus, that they cannot be termed as "reacting groups".

The results will be published more fully in further papers.

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